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**Satellitome characterization in flour beetles of  
the genus *Tribolium***

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### KARAKTERIZACIJA SATELITOMA KUKACA BRAŠNARA IZ RODA *TRIBOLIUM*

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**Doktorski rad je izrađen u:** Laboratoriju za nekodirajuće DNA, Zavoda za molekularnu biologiju, Instituta Ruđer Bošković u Zagrebu

**Mentor:** dr.sc. Brankica Mravinac

#### **Kratki sažetak doktorskog rada:**

Satelitne DNA su uzastopno ponavljajuće, nekodirajuće DNA sekvence prisutne u eukariotskim genomima. Kukci brašnari roda *Tribolium* odlična su modelna skupina za proučavanje satelitnih DNA zbog njihove visoke zastupljenosti u genomima. U ovom radu eksperimentalnim i bioinformatičkim pristupima sveobuhvatno su karakterizirani satelitomi vrsta *Tribolium freemani*, *Tribolium madens*, *Tribolium confusum* i *Tribolium brevicornis*, te je ukupno identificirano 533 satelitnih DNA. Provedene komparativne analize satelitoma doprinijele su razumijevanju građe genoma i evolucije satelitnih DNA koje u njima dominiraju, potencijalno utječući na specijaciju vrsta roda *Tribolium*.

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### SATELLITOME CHARACTERIZATION IN FLOUR BEETLES OF THE GENUS *TRIBOLIUM*

Damira Veseljak

**Thesis performed at:** Laboratory of non-coding DNA, Division of Molecular Biology, Ruđer Bošković Institute in Zagreb

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#### Short abstract:

Satellite DNAs are tandemly repeated noncoding DNA sequences found in the eukaryotic genomes. The flour beetles of the genus *Tribolium* represent a great model for satellite DNA research due to the abundance of satellite DNAs in their genomes. In this work, implementing an experimental and bioinformatics approach, satellitomes of the species *Tribolium freemani*, *Tribolium madens*, *Tribolium confusum* and *Tribolium brevicornis* were comprehensively characterized and 533 satellite DNAs were identified. Comparative analyses of the satellitomes contributed to the understanding of the genome structure and the evolution of satellite DNAs as their dominant fraction, potentially contributing to the speciation of the *Tribolium* species.

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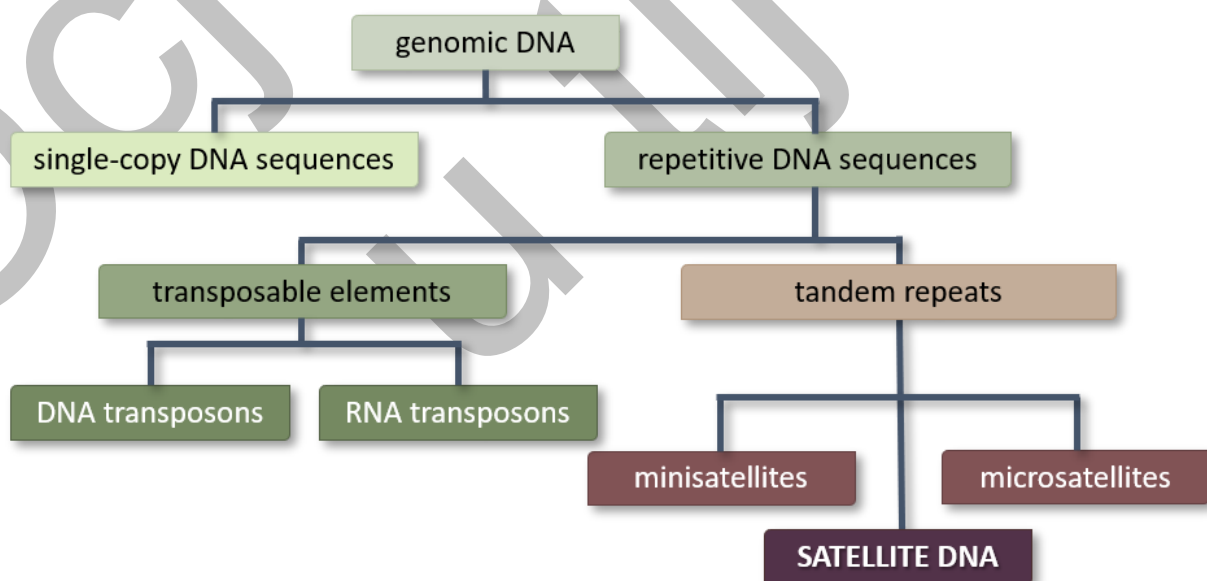
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# 1. INTRODUCTION

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## 1.1. Satellite DNA

Eukaryotic genomes can vary greatly in size, from the small 12 megabase (Mb) genome of the yeast *Saccharomyces cerevisiae* (Wei et al. 2007) to the 160 gigabase (Gb) largest known eukaryotic genome of the fern *Tmesipteris oblancheolata* subsp. *linearifolia* (Fernández et al. 2024). The composition of the DNA sequences that make up the eukaryotic genomes is very complex and varies between different genomes. Apart from single-copy genes, which code for specific proteins and do not show homology with other genomic sequences, eukaryotic genomes are rich in different types of repetitive DNA (Figure 1), often accounting for more than half of the total genomic content. The two categories into which repetitive DNA can be generally sorted are: (i) interspersed repetitive sequences consisting mostly of transposable elements (TE), namely DNA and RNA transposons, and (ii) tandem repeats (TR) (Biscotti et al. 2015; Liao et al. 2023). The most abundant of tandemly repeated DNA sequences are satellite DNAs (satDNAs). Their repeat unit, often referred to as a monomer, is tandemly repeated in head to tail fashion forming long genomic arrays. SatDNAs are mainly associated with the heterochromatic areas of the eukaryotic genomes such as (peri)centromeres and (sub)telomeres (Plohl et al. 2012), but can also be found in the euchromatin (Pavlek et al. 2015; Cabralde-Mello et al. 2023; Rico-Porras et al. 2024). Other types of TRs in eukaryotic genomes that differ from satDNAs by their monomer length are minisatellites and microsatellites (reviewed in Garrido-Ramos 2017). Microsatellites, also known as short tandem repeats (STR), are characterized by a repeat unit length of less than 10 base pairs (bp), whereas minisatellites have longer monomeric units of more than 10 bp (Garrido-Ramos 2017). In contrast to the short repeat unit sizes of microsatellites and minisatellites, monomers of satDNAs exhibit larger variations in monomer length, ranging from a few tens of bp to several thousand bp even in one genome (Gálvez-Galván et al. 2024).



**Figure 1.** Classification of the repetitive DNA sequences in the eukaryotic genomes.

### 1.1.1. Detection of satellite DNAs

The earliest instances of experimentally observed satDNAs were obtained in 1961 by Kit in the genomes of mice and guinea pigs and by Sueoka in the genomes of two species of crab (*Cancer borealis* and *Cancer irroratus*) by density centrifugation in cesium chloride (CsCl) gradients (Figure 2). After centrifugation, the satDNAs formed a band separate from the rest of the genomic DNA, corresponding to the different buoyant densities and G-C contents (Kit 1961; Sueoka 1961). However, the authors were not yet able to explain these phenomena, theorizing the possible contamination of the DNA with proteins or foreign DNA (Sueoka 1961). Therefore, the term “satellite” was initially used to describe the band that differs from the remaining genomic DNA in the gradient of CsCl. Further advances in the field of satDNAs were made following DNA renaturation experiments on the satellite band of mouse DNA. The DNA renaturation rate of the satellite was faster than that of the genomic band, leading to the conclusion that the nucleotide sequence of the mouse satellite band is made up of short fragments repeated numerous times with little variation (Figure 2) (Waring and Britten 1966).

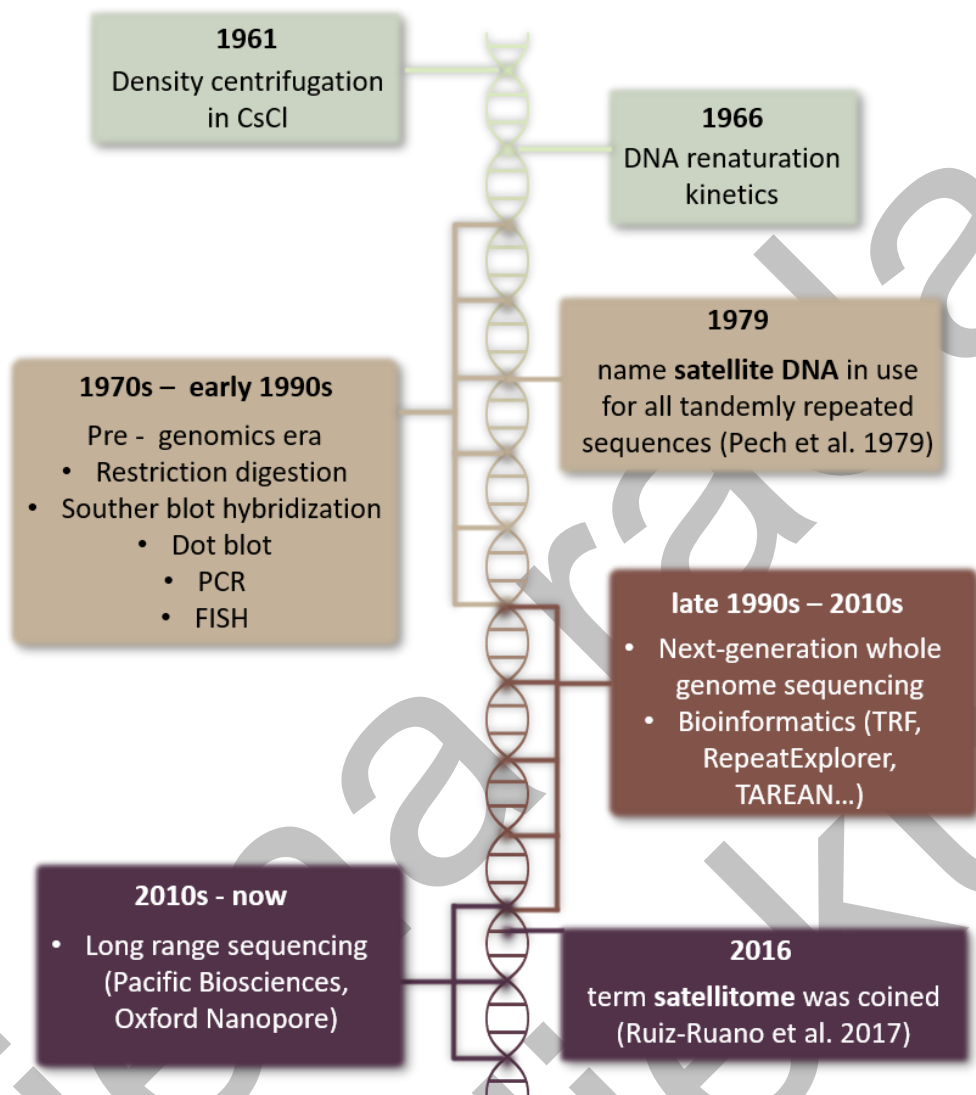
With the discovery of restriction endonucleases (Smith and Wilcox 1970), which enabled the cleavage of DNA at the specific nucleotide motif, the genomic organization of satellites could be further investigated. When mutation occurs at the restriction site within the monomer of the satDNA or due to incomplete restriction digestion, electrophoretic separation of the fragments on the agarose gel yields a ladder-like pattern, demonstrating the tandem organization of satellites (Singer 1982). The specific bands could be isolated from the agarose gel and, due to advances in molecular cloning, amplified. This was followed by, at the time, newly developed methods. Southern blot hybridization (Southern 1975) was used to explore the variation of monomer sequences, dot-blot hybridization for rough estimates of genomic abundance, and sequencing (Maxam and Gilbert 1977) to determine the nucleotide composition of satellite monomers (Singer 1982; Garrido-Ramos 2017). Even though the term satellite was initially used only for DNA separated by ultracentrifugation, the similarity of the repeated sequences discovered by restriction digestion with the properties of the distinct satellite bands, warranted the term to become synonymous with tandemly repeated genomic DNA (Figure 2) (Pech et al. 1979).

Further improvements in the methodology of satDNA detection and characterization were achieved by implementing fluorescence *in situ* hybridization (FISH) and polymerase chain reaction (PCR) into the research workflow. Interestingly, one of the first protocols for *in situ* hybridization experiments was developed for chromosomal localization of the mouse satellite, which was discovered by Kit (Pardue and Gall 1970). However, as the method used radioactivity for cytogenetic probe labelling, it only became widely used with the advent of fluorescent hybridization probes (Gall 2016) (Figure 2). However, using previously described classical molecular biology methods, it was only possible to identify satDNAs that dominate the genomes of the species, often just one or two per organism.

With the technological advances of the late 1990s and early 2000s, novel methods for sequencing DNA, known as Next Generation Sequencing (NGS) were developed, enabling easier and more cost-effective sequencing of genomes (Figure 2) (Hu et al. 2021). Although this resulted in a larger number of genomic assemblies, repetitive fractions of these genomes remained underrepresented. Nevertheless, new algorithms for detection of satDNAs from the genomic assemblies, such as Tandem Repeats Finder (TRF) were developed (Figure 2) (Benson 1999). However, the real breakthrough in satDNA research was the introduction of graph-based clustering of short reads obtained by NGS which groups the reads together according to their similarities and enables *de novo* identification of repeats without the need for prior genome assembly (Novák et al. 2010). The algorithm was later implemented as a collection of software tools in the web interface of the Galaxy platform under the name RepeatExplorer (Figure 2) (Novák et al. 2013; Jalili et al. 2021) and further improved as RepeatExplorer2 (Novák et al. 2020). Of particular importance for the identification of satDNAs was the development of the new module of RepeatExplorer, which specializes in the detection of satDNAs, called Tandem Repeat Analyzer (TAREAN) (Figure 2) (Novák et al. 2017). TAREAN enabled the characterization of multiple, high or low-copy, satDNA families in the genomes by providing consensus sequences of the potential new satDNAs (Novák et al. 2017). By using graph-based clustering it became possible to detect numerous satDNA families in eukaryotic genomes (Heckmann et al. 2013; Ruiz-Ruano et al. 2017). Consequently, the new term satellitome was coined, describing the total collection of satDNA families in the genome after the detection of 62 different satDNAs in the genome of the migratory locust *Locusta migratoria* (Figure 2) (Ruiz-Ruano et al. 2016). As the TAREAN pipeline improves and becomes more precise, the search for satDNAs becomes more productive, with several hundred different satDNA families detected in numerous organisms (Cuadrado et al. 2023; Mora et al. 2023; da Silva et al. 2023). Even though the TAREAN pipeline enabled *de novo* identification of a plethora of satDNAs and provided their estimated genome abundance, it does not provide clues for determining their genomic context such as their chromosomal localization or length of the satDNA arrays.

Currently, research of satDNAs has again been strongly driven forward by the introduction of long-read sequencing by Oxford Nanopore Technologies (ONT) and Pacific Biosciences High Fidelity (PacBio HiFi) sequencing (Figure 2). Long, highly accurate reads enabled more complete genome assemblies with the inclusion of the repeat regions (Miga et al. 2020; Altemose et al. 2022) that provided insight into the genomic organization of satDNAs. Also, even using just the raw PacBio HiFi reads can be beneficial for investigating the evolution of satDNA families in related species (Peona et al. 2023) because of the 99.8% fidelity and more than 13.5 kb read length (Wenger et al. 2019).





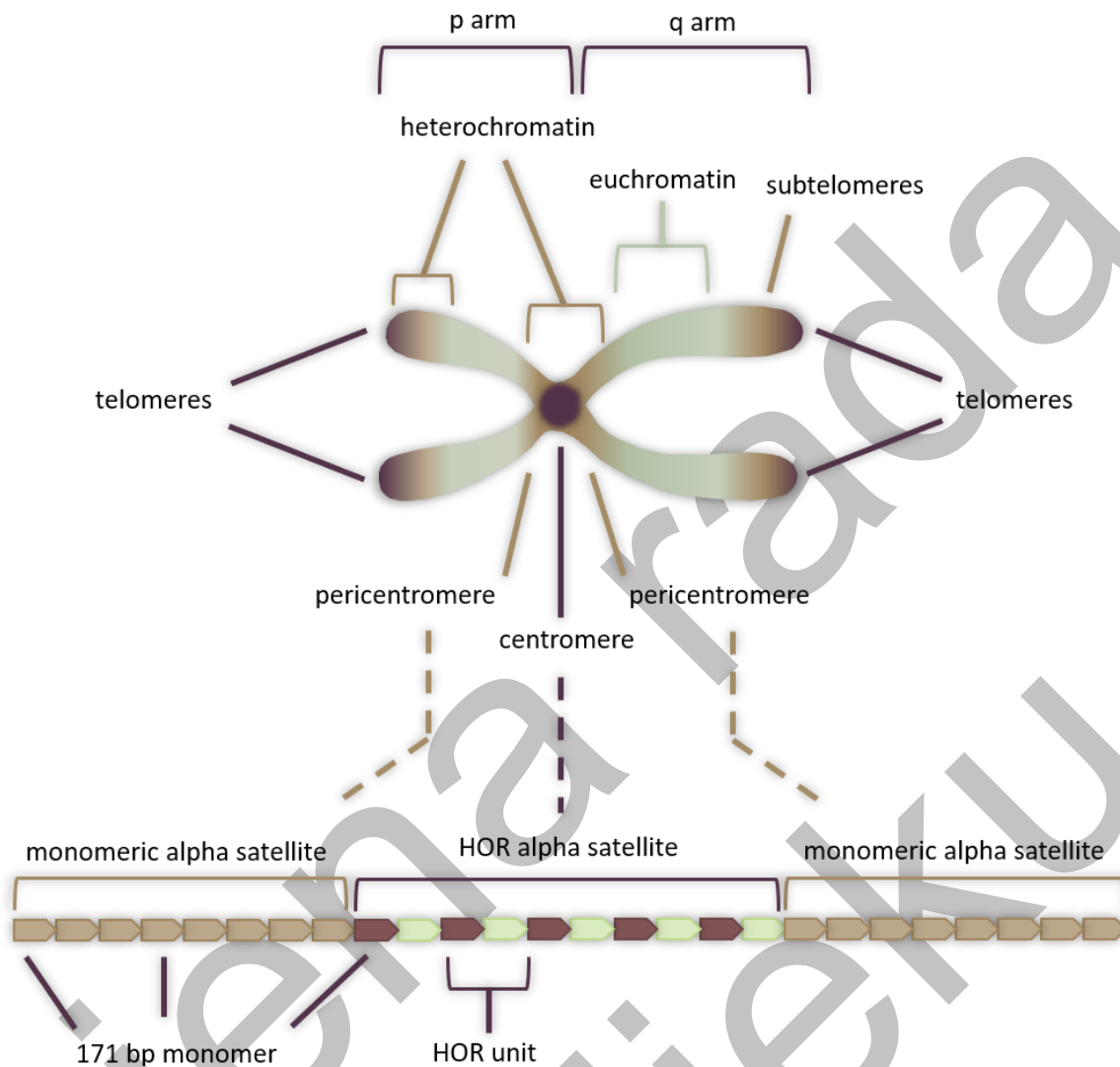
**Figure 2.** Milestones in the development of methods for the discovery and characterization of satellite DNAs in eukaryotic organisms.

### 1.1.2. Structure and organization of satellite DNAs

Tandemly repeated satDNA monomers in some cases span more than a megabase (Mb) (Altemose et al. 2022; Macas et al. 2023). The preferred length of a satDNA basic repeat unit is between 150 – 180 bp and 300 – 360 bp, linking the length of monomer to the length necessary to encircle the nucleosome one or two times (Henikoff et al. 2001). Nevertheless, satDNAs show a large span of monomer sizes, from just several dozen bp in *Petunia hybrida* (Alisawi et al. 2023) to almost 6 kb long pericentromeric satDNA of the potato *Solanum tuberosum* (Stupar et al. 2002). As the satDNA arrays consist of numerous monomers that could have different nucleotide sequences despite their low divergence, the consensus sequence of satDNAs based on available monomer variants is usually generated (Willard and Wayne 1987).

Except for the neatly repeated satDNA monomers, the nucleotide sequence of the monomer or the organization of the monomers in an array can be complex and form higher order repeats (HOR). The term HOR refers to the organizational pattern of satDNA in which, in contrast to a classical satDNA array, a repeat unit is of multimeric origin. The HOR unit is conserved in its structure, with monomers of the same type always occupying the same position in the HOR after undergoing simple amplification and fixation (Willard 1991). The satDNA with the best defined HORs is the human alpha satellite, whose monomer unit length is 171 bp. Although its monomers can be arranged in classical satDNA arrays, with repeats that are on average 70% identical and arranged in no recognizable pattern, these arrays are primarily concentrated in pericentromeric regions on chromosomes (Figure 3) (Wevrick et al. 1992). However, the centromeric arrays are organized as HORs, which are homogeneous, array- and chromosome-specific, with monomers sharing the same position in the HOR being the most similar. The satDNA itself has twelve different defined consensus sequences (J1, J2, D1, D2, W1, W2, W3, W4, W5, M1, R1 and R2) which are subdivided into five suprachromosomal groups (SF1 – SF5). As an illustration of suprachromosomal groups, SF1 is dimerically organized with alternating J1 and J2 variants and is present on 9 chromosomes, but with variations in the organization and number of the monomers specific to different chromosomes (Alexandrov et al. 2001; McNulty and Sullivan 2018). For example, the SF1 variant of the alpha satellite present on chromosome 1 has a simple dimeric HOR structure of J1 and J2 monomers repeated respectively and is 340 bp long (Figure 3) (Carine et al. 1989). The HOR organization of satDNA has also been detected in non-primates, examples of which include SF05 satDNA superfamily of the grasshopper *Pyrgomorpha conica* (Ruiz-Ruano et al. 2018) and Ymin satDNA specific for Y chromosome of the house mouse *Mus musculus* (Pertile et al. 2009).

The monomer unit of satDNA by itself can be of complex structure. An example of this is satDNA families with several different monomer types that stem from the same sequence. In the beetle *Chrysolina carnifex*, the satDNA CCAH has three different monomer unit types, two of which have dimeric (CCAH-477) and trimeric (CCAH-633) HOR structure (Palomeque et al. 2005). More recently, the satDNA PSAT6 of the garden petunia *Petunia hybrida* was described to have two different subunits of 38 and 39 bp, which have a similarity of 65-70% and together form a dimeric HOR unit of 78 bp (Alisawi et al. 2023).



**Figure 3.** Schematic view of human chromosome zoomed in to the organization of pericentromeric monomeric alpha satDNA arrays and centromeric HOR arrays specific for SF1 suprachromosomal family on chromosome 1.

The HORs of the monomeric units can be even more complex and can often be composed of inverted repeats that vary in length. The monomer of the aptly named partially inverted repeat or PIR satDNA found in the chicken *Gallus gallus domesticus* genome, is 1430 bp long, organized in central (~1000 pb) and a flanking (~430 bp) region. The central region is bordered on each side by an 86 bp long inverted repeat (Li et al. 2007). In the parasitic wasp species, namely *Diadromus collaris*, the monomer of the satDNA is 512 bp long and consists of two duplicated regions bridged by a 169 bp long sequence consisting of one sequence and a corresponding inverted repeat (Rojas-Rousse et al. 1993). Furthermore, in the group of Asian subtropical lady slipper orchids (*Paphiopedilum* subgenus *Parvisepalum*), the SatA has four different subfamilies, each of them containing an inverted repeated region that is speculated to form hairpin loop motifs (Lee et al. 2018). It has been proposed that the

inverted repeats form dyad structures with potential roles in heterochromatin condensation and protein binding (Rojas-Rousse et al. 1993; Mravinac et al. 2004).

SatDNA abundance in the different genomes varies greatly, ranging from less than 0.1% to more than 50% of the total genomic content (Cabral-de-Mello et al. 2021; Mascagni et al. 2022; Mora et al. 2023). Even though the satellitomes consist of multiple different satDNA families, it is often the case that one satDNA dominates the genome. For example, 226 different satDNAs comprise the satellitome of the frog *Proceratophrys boiei*. However, a single satDNA PboSat01-176 accounts for 93.62% of the satellitome content, as it is present in large blocks on the centromeric regions of all chromosomes in the complement (da Silva et al. 2023). For this reason, it was subsequently proposed that it could serve as a chromosomal centromeric marker (da Silva et al. 2024). The additional examples of major satDNA that is distributed across all the chromosomes include the tenebrionid insects *Alphitobius diaperinus* and *Tenebrio molitor* (Bruvo et al. 1995), and the centromeric satDNA of *Arabidopsis thaliana* (Naish et al. 2021). Furthermore, satDNAs can be positioned on fewer chromosomes (Gutiérrez et al. 2023; Toma et al. 2023; Kretschmer et al. 2024), but they can also be chromosome specific. In the plant *Muscari comosum*, satDNA MCSAT was found to be exclusively present on one chromosome pair (De La Herrán et al. 2001). It is also common for satDNAs to be present only on sex chromosomes, as seen with X-chromosome specific satDNA of the northern red muntjac *Muntiacus muntjak vaginalis* (Bogenberger et al. 1982) and different satDNAs of the fish *Megaleporinus elongatus* (Crepaldi and Parise-Maltempi 2020). The chromosome specific satDNAs can be used as markers for the differentiation of chromosomes in cytogenetic experiments.

Historically, because of the robustness of classical molecular biology methodology used for the detection of satDNAs, only the most abundant satDNAs were detected. Those satDNAs were usually positioned in the (peri)centromeres and (sub)telomeres, cementing the belief that satDNAs could be found exclusively in heterochromatin. However, with the advancements in sequencing technologies that lead to more complete genome assemblies, it became apparent that satDNAs could also be found in euchromatin. In the red flour beetle *Tribolium castaneum*, nine different euchromatic satDNA families named Cast1-9 were characterized. The satDNAs showed efficient propagation in euchromatin with the possible role of regulation of gene expression (Pavlek et al. 2015). Furthermore, it was discovered that the satDNAs of the bug *Oxycarenus hyalinipennis* are present only in euchromatin (Cabral-de-Mello et al. 2023). In the beetle *Chrysolina americana*, more abundant satDNAs were found present in large blocks in pericentromeric regions, while the low-copy number satDNAs were found scattered thorough euchromatin (Rico-Porrás et al. 2024). Comparative satellitome analysis of the two species of kissing bugs from the genus *Triatoma*, *Triatoma delpontei* and *Triatoma infestans* revealed that the two insects share much of the satDNA families, but with different genomic positions and abundance. The common occurrence for the shared satDNAs was to be positioned in heterochromatic regions in one species but found only in euchromatin in the other (Mora et al. 2023).

### 1.1.3. Function of satellite DNAs

Throughout history, the repetitive fraction of the eukaryotic genomes, and more specifically satDNAs, were referred to as “junk” DNA since they have no specific protein-coding function, and they also inflate heterochromatic portions of the genome (Ohno 1972). However, even at the early stages of satDNA research several hypotheses were proposed to explain potential satDNA function, such as role in chromosome organization by stabilizing centromeres and telomeres, facilitating correct chromosome pairing and segregation, and their role in speciation and evolution (John and Gabor Miklos 1979). But the potential roles of the previously considered “junk” DNA came into the spotlight with the completion of The Encyclopedia of DNA Elements or ENCODE (Consortium 2012) after which the death of the term has been ceremoniously announced (Pennisi 2012). The functions of satDNAs are currently being vastly studied. Although various studies indicate many different roles of satDNA, there is still no universal function of satDNA that is conserved among the species studied.

The most notable function is the role of satDNA in the formation of active centromeres. The previously mentioned alpha satellite forms the centromeres of primate chromosomes (Thakur et al. 2021). One of the monomeric units of the dimeric HORs of the alpha satellite, which can be grouped into SF1 and SF2, contains within its nucleotide sequence the CENP-B box that interacts with the CENP-B protein of the kinetochore complex (Henikoff et al. 2015). The mammalian CENP-B protein is the only protein of the complex known to recognize a specific DNA sequence of the centromere (Masumoto et al. 1989). In addition to primate genomes, the CENP-B box-like motif has also been found in the satDNA sequences of numerous species, including those of nematodes of the genus *Meloidogyne* (Despot-Slade et al. 2021), two Gerbilinae species (Uno et al. 2023) and mouse (Kipling et al. 1995). The transcription of the alpha satellite has also been researched. It was shown that the RNA transcripts of the alpha satellite have a role in engaging the proteins CENPC1 and INCENP of the centromere nucleoprotein complex leading to proper kinetochore assembly, therefore taking part in proper chromosome segregation (Wong et al. 2007).

The transcription of different satDNAs has been studied in multiple different organisms. In the yeast *Schizosaccharomyces pombe*, transcripts from the centromeric repeats containing TRs were found to be involved in the formation and maintenance of heterochromatin via RNA interference (RNAi). The small siRNAs interact with an Argonaute protein to form an RNA-induced transcriptional silencing complex (RITS) (Volpe et al. 2002). The RITS complex then promotes the methylation of HSK9 histone, one of the hallmarks of inactive heterochromatin, which in turn leads to the formation of additional siRNAs through the recruitment of various proteins, representing a positive feedback loop (reviewed in Johnson and Straight 2017). The processing of satDNAs into siRNAs has also been observed in the sugar beet (Zakrzewski et al. 2011) and rice (Lee et al. 2006) where it has been hypothesized to be involved in the maintenance of heterochromatin.

In humans, the expression of satDNA called satellite-III has been connected to the stress response. It was determined that the transcription factor Heat Shock Factor 1 (HSF1), in addition to transcriptional activation of various stress response proteins, activates the transcription of satellite-III (Jolly et al. 2004; Col et al. 2017). Differential expression of various repetitive elements was studied in early human development, and it was discovered that numerous satDNAs are expressed during the pre-implantation period and highly expressed during the 4-cell stage with specific patterns. However, some of the satDNAs were under expressed or not expressed at all (Yandlm and Karakülah 2019). The link between heat-induced stress and the expression of satDNAs was discovered in the red flour beetle *T. castaneum*. The transcription rate of the major satDNA TCAST1, located in the (peri)centromeric heterochromatin, is increased in relation to heat shock, which in turn leads to more epigenetically repressed heterochromatin (Pezer and Ugarković 2012). Further studies revealed that the transcripts of TCAST1 which is dispersed in the gene coding regions of the euchromatin, repress the activity of genes located nearby under heat stress condition (Felicciello et al. 2015). In addition, the differential expression of the TCAST1 transcription was detected during the different developmental stages of the beetle (Sermek et al. 2021).

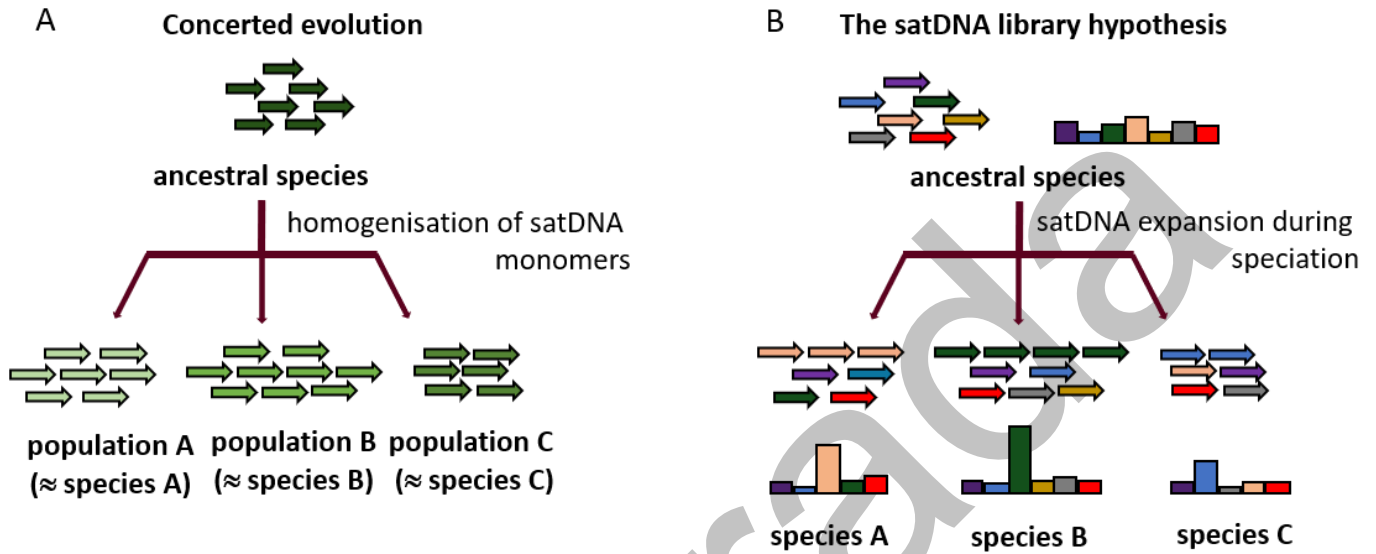
Human satDNAs have been linked with tumorigenesis, with an association with genomic instability and improper mitosis (reviewed in Ferreira et al. 2015). In advanced metastatic prostate cancer, alpha satellite DNA on chromosomes 2, 3, 4, 15 and 20 and satellite-III were shown to be excessively overamplified (Ariffen et al. 2024). The presence of long non-coding RNAs (lncRNAs) derived from the pericentromeric satDNAs HS2/HS3 was detected in large numbers in the cancer-associated fibroblasts (CAFs) of human adenocarcinoma and was associated to the regulation of inflammatory genes (Erukashvily et al. 2023). In addition, satellite 2 DNA has been proposed as a potential biomarker for the detection of cancer, as it has been detected in substantial amounts in the blood plasma of various cancer patients (Özgür et al. 2021).

The role of satDNAs in speciation has also been studied. It is well known that satDNAs are often the most divergent regions of the genomes of some related species (Robledillo et al. 2020; Schmidt et al. 2024). In the study of *Drosophila melanogaster* and *Drosophila simulans* hybrids, it was found that the 359 bp long satellite from the X chromosome of *D. melanogaster*, which is not present in the genome of *D. simulans*, upon paternal inheritance causes delayed chromatin during early embryogenesis of the hybrids. Ultimately, lagging chromatin prevents the separation of chromosomes during mitosis, leading to hybrid incompatibility (Ferree and Barbash 2009). Also, in *Drosophila*, pericentromeric satDNAs are known to be responsible for the grouping of heterologous chromosomes into a single nucleus forming chromocenters, the disruption of which can lead to the loss of chromosomes and eventual cell death (Jagannathan et al. 2018). Additional studies of hybrids of *D. melanogaster* and *D. simulans*/*D. mauritania* revealed that the divergence of satDNAs leads to defective chromocenter assembly (Jagannathan and Yamashita 2021). Research on *Drosophila* hybrids indicates that differentiation of satDNAs ultimately leads to reproductive isolation of the species.

#### 1.1.4. Evolution of satellite DNAs

SatDNAs are widely considered as the fastest evolving fraction of eukaryotic genomes. Despite this, monomers within satDNA arrays are homogenous with typically low sequence divergence. The reason for this is that satDNA monomers do not evolve independently within the array. When the mutation in sequence occurs, it spreads throughout the other repeat units in an array, or it disappears entirely. The homogenization of the sequences occurs due to the mechanism of non-reciprocal sequence transfer, while the fixation is the result of the random transfer of genetic material during meiosis and sexual reproduction (Dover 1986). Homogenization and fixation are part of the evolutionary process known as molecular drive (Dover 1982). The process of molecular drive explains the concept of concerted evolution in which a satDNA monomer variant homogenizes and fixates in the population, becoming different from the variant in another population which can ultimately lead to speciation. The concerted evolution results in satDNA monomers with higher sequence identity within the species than between the monomers of different species (Figure 4A) (Dover 1986).

The second important concept of the satDNA evolution is the “library” hypothesis. The hypothesis explains that there might be shared satDNA families in the genomes of related species. The changes in satDNAs between species include primarily variations in the copy number due to differential amplification. To be more precise, the satDNA family, which originated from the ancestral species, can experience a burst in the number of monomers becoming major satDNA in one of the daughter species, but remain low-copy in others. This leads to the species-specific satDNA profile (Figure 4B) (reviewed in Plohl et al. 2012). The satDNA library hypothesis was first suggested by Fry and Salser in 1977 by exploring the H $\alpha$  Satellite DNA from the kangaroo rat *Dipodomys ordii* that was found to be conserved among different rodent species. The first experimental proof for the concept was provided by Meštrović et al. in the insect genus *Palorus*. The satDNA libraries were further confirmed in various species including root-knot nematodes from the genus *Meloidogyne* (Meštrović et al. 2006), Characidae fish (Utsunomia et al. 2017), grasshoppers from the genus *Schistocerca* (Palacios-Gimenez et al. 2020) and different genera of sturgeons in which satDNAs were conserved even though the species diverged more than 100 Mya (Robles et al. 2004).



**Figure 4.** Schematic view of the evolutionary concepts of concerted evolution (A) and the satDNA library hypothesis (B).

Homogenization and propagation of satDNA monomers is widely considered to be driven by a non-reciprocal transfer of sequences. The mechanisms involved are unequal crossover, gene conversion, rolling circle replication, and transposition (Dover 1986). Unequal crossover is an important mechanism for the spread of satDNAs. Since the monomers of satDNAs are tandemly repeated, their arrays have many homologous points that offer hot spots for recombination. By the mechanism of unequal crossover, parts of the satDNA arrays could be duplicated or deleted, fixating the more similar sequences in the genomes and altering both the donor and recipient DNA strands (Smith 1976). Furthermore, the high homology of satDNA arrays also makes them suitable for unidirectional transfers of genetic material using the mechanism of gene conversion. More specifically, the transfer of genetic material from a donor strand to a recipient strand takes place, with the recipient sequence being completely replaced by the donor sequence. The result is the number of monomers, or differently positioned satDNA arrays, that share the same mutations (Dover 1986). Both unequal crossover between sister chromatids and gene conversion have been identified as factors contributing to the evolution of human alpha satellite DNA (Waye and Willard 1986; Warburton and Willard 1992; Alexandrov et al. 1993; Mashkova et al. 1998), centromeric satDNAs in *Arabidopsis* species (Wlodzimierz et al. 2023) and *Rsp* locus satDNA of *D. melanogaster* (Khost et al. 2017).

The rolling circle replication generates extrachromosomal circular DNAs (eccDNAs) by homologous recombination between direct repeats, which then reinsert into the genome via homologous or non-homologous recombination, leading to deletion or expansion of satDNA arrays (reviewed in Cohen and Segal 2009). First evidence of the satDNA eccDNAs was found in *D. melanogaster* (Cohen et al. 2003) but they were later confirmed in two additional *Drosophila*



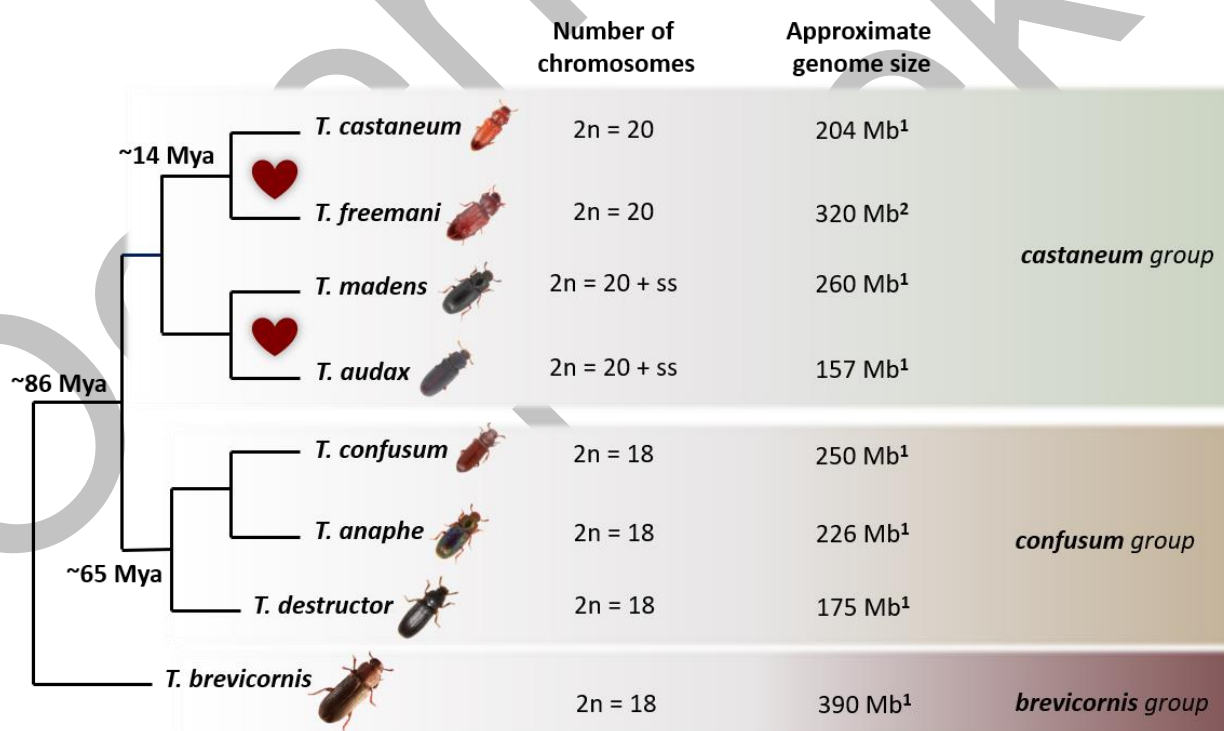
species (Sproul et al. 2020) and ten species of higher plants from various genera (Navrátilová et al. 2008).

Another mechanism involved in propagation of satDNA is transposition. It is known that many satDNAs share homologies with different transposable elements. This can be observed in the pea *Pisum sativum* genome in which the 3' untranslated region (UTR) of the Tat lineage of LTR-retrotransposon carries various different short TRs that are also found tandemly repeated as satDNA arrays in different genomic regions. The short TRs could be moved through the genome by transposition and subsequently amplified, therefore providing origin for the tandemly repeated arrays (Macas et al. 2009). The additional examples of satDNAs derived from TEs include satellite 1 of the African clawed frog *Xenopus laevis* that showed sequence homology with SINE elements (Pasero et al. 1993) and maize satDNAs CRM1TR and CRM4TR that originated from centromeric retrotransposons (Sharma et al. 2013). More recently, it has been determined that the satDNA Pch-Sat, which can be found in the genomes of the mollusk *Patella caerulea* and its relatives, shares homology with the truncated segment of the Nin-SINE transposable element, suggesting the tandemization of the segment as the origin of Pch-Sat (Petraccioli et al. 2024). Furthermore, the analysis of genome assembly of the hybrid plant *Populus tremula* x *Populus alba* revealed that the satDNA PtaM147 has its origin in the LTR-retrotransposons (Zhou et al. 2023). New and improved genome assemblies enable us to further broaden our understanding of co-existence and dynamics between the transposable elements and satDNAs.

## 1.2. The genus *Tribolium*

The genus *Tribolium* belongs to the insect order Coleoptera which is the most numerous animal order occupying the wide range of ecological habitats. It contains 387,000 described species, making it 40% of all described insect species and 25% of all described animal species (Stork 2018). Inside the order Coleoptera, the family Tenebrionidae or darkling beetles consists of around 20,000 described species, including the flour beetles of the genus *Tribolium*.

The genus *Tribolium* comprises over 30 different flour beetle species. Ten of the species are pests living in the storages of agricultural products, with several of them, including *T. castaneum* and *Tribolium confusum*, being cosmopolitan (Angelini and Jockusch 2008). The species of the genus *Tribolium* are commonly divided into five different species groups, according to their morphology and their original geographical distribution. The groups in question are the *castaneum* group, the *confusum* group, the *brevicornis* group, the *alcine* group and the *myrmecophilum* group (Table 1, Figure 5) (Hinton 1948). The genus *Tribolium* is considered to be evolutionarily old, with its age estimated to be over 100 Mya. Despite its evolutionary old age, the genus also consists of sibling species for which speciation occurred much later. An example of this is the sibling species pair *T. castaneum* and *Tribolium freemani*, which diverged around 14 Mya (Figure 5) (Ramesh et al. 2021). The variety of divergence times within the genus *Tribolium* makes it an excellent platform for different evolutionary studies.



<sup>1</sup>Alvarez-Fuster et al. 1991

<sup>2</sup>Volaric et al. 2022

**Figure 5.** Phylogenetics of the genus *Tribolium* according to Angelini and Jockusch 2008 and Ramesh et al. 2021 with chromosome number and genome size. Hearts mark the pairs of sibling species.

**Table 1.** Species of the genus *Tribolium* with their geographical distribution according to Hinton 1948 and Angelini and Jockusch 2008.

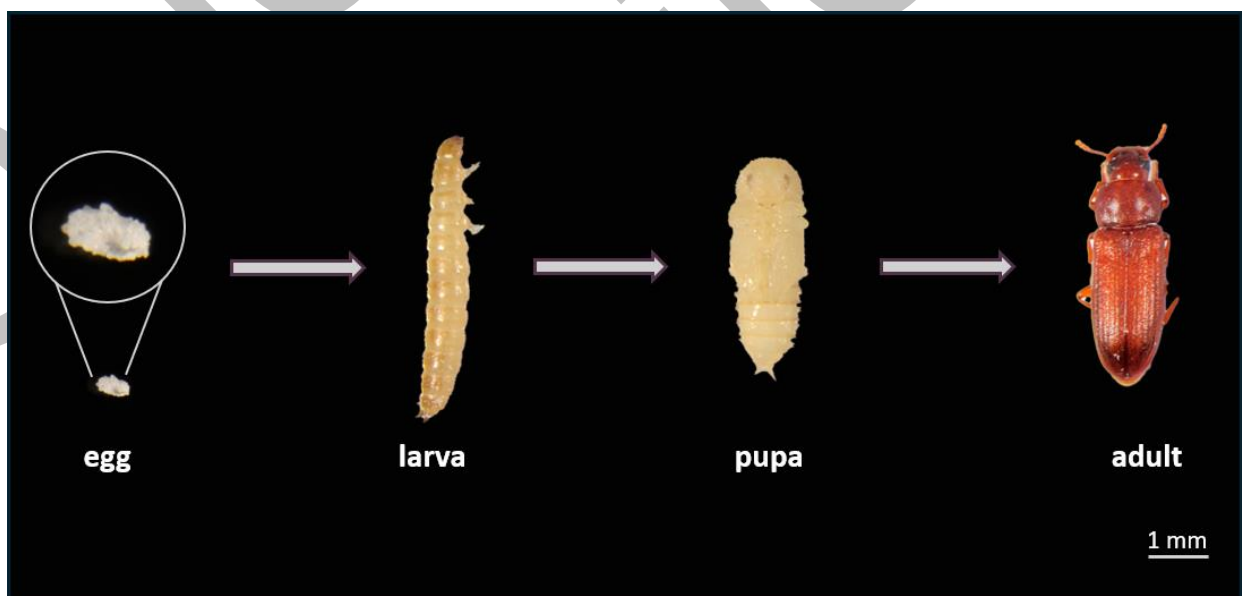
	The species of the genus <i>Tribolium</i>	Geographical distribution
<b>castaneum group</b> South and Southeast Asia	<i>T. apiculum</i> Neboiss (1962)	Australia
	<i>T. audax</i> Halstead (1969)*	North America <sup>1</sup>
	<i>T. caledonicum</i> Kaszab (1982)	Lifou (New Caledonia)
	<i>T. castaneum</i> Herbst (1997)*	Cosmopolitan <sup>1</sup>
	<i>T. cylindricum</i> Hinton (1948)	Malay peninsula, Indonesia, Borneo, Philippines
	<i>T. freemani</i> Hinton (1948)*	Kashmir, Japan <sup>1</sup> , Brazil <sup>1</sup>
	<i>T. madens</i> Charpentier (1825)*	Cosmopolitan <sup>1</sup>
	<i>T. parki</i> Hinton (1948)	Bali, Larat (Indonesia)
	<i>T. politum</i> Hinton (1948)	Doerian (Indonesia)
	<i>T. waterhousei</i> Hinton (1948)	Queensland, New South Wales
<b>confusum group</b> Africa	<i>T. anaphe</i> Hinton (1948)*	Central Africa
	<i>T. arndti</i> Grimm (2001)	South Africa
	<i>T. bremeri</i> Grimm (2001)	South Africa
	<i>T. confusum</i> Jacquelin du Val and Fairmaire (1868)*	Cosmopolitan <sup>1</sup>
	<i>T. destructor</i> Uyttenboogaart (1934)*	Cosmopolitan <sup>1</sup>
	<i>T. downesi</i> Hinton (1948)	Mali, Sudan, Chad
	<i>T. ferreri</i> Grimm (2001)	Gambia
	<i>T. indicum</i> Blair (1930)	Central Africa, Saudi Arabia <sup>1</sup> , Iran <sup>1</sup> , India <sup>1</sup>
	<i>T. risbeci</i> Lepesme (1943)	Senegal
	<i>T. semele</i> Hinton (1948)	Mali, Mauritania, Sudan, Chad
	<i>T. semicostata</i> Gebien (1910)	Kenya
<i>T. sulmo</i> Hinton (1948)	Ethiopia, Gambia, Ghana	
	<i>T. thusa</i> Hinton (1948)	Chad, South Africa, Botswana, Namibia
<b>brevicornis group</b> North and South America	<i>T. brevicornis</i> Leconte (1859)*	California
	<i>T. carinatum</i> Hinton (1948)	Argentina
	<i>T. gebieni</i> Uyttenboogaart (1934)	Paraguay
	<i>T. linsleyi</i> Hinton (1948)	Mexico
	<i>T. parallelus</i> Casey (1890)	Western North America
	<i>T. setosum</i> Triplehorn (1978)	Arizona
<b>alcine group</b> Madagascar	<i>T. alcine</i> Hinton (1948)	Madagascar
	<i>T. ceto</i> Hinton (1948)	Madagascar
	<i>T. quadricollis</i> Fairmaire (1902)	Madagascar
<b>myrmecophilum group</b> Australia	<i>T. antennatum</i> Hinton (1948)	Queensland
	<i>T. myrmecophilum</i> Lea (1904)	Victoria

<sup>1</sup> The species were possibly introduced by human activity.

\* Eight species used in research studies.

### 1.2.1. Red flour beetle *Tribolium castaneum*

The red flour beetle *T. castaneum* is the representative of the genus *Tribolium* and one of the most studied insect laboratory organisms, second only to the most common insect model organism *D. melanogaster*. Due to the accelerated evolution of *Drosophila* and the more basal position of the genus *Tribolium* in the phylogenetic tree, *T. castaneum* is the better representative of insects overall (Richards et al. 2008). It is widely used in various research fields, including population genetics, functional genomics, and developmental and evolutionary biology (Kumar et al. 2018). The factors that have significantly contributed to the popularity of *T. castaneum* in research are the minimal maintenance required for rearing *T. castaneum* cultures in laboratory conditions and the numerous offspring it produces. Furthermore, *T. castaneum* has holometabolous development and passes through complete metamorphosis that includes four developmental stages (egg, larva, pupa and adult) in about 30 days, which makes it a great model for developmental studies (Figure 6). The long-standing presence of *T. castaneum* in research studies resulted in several hundred different genetic mutants that are available for the scientific community (Brown et al. 2009). The extensive research performed on *T. castaneum* was compiled in the comprehensive database containing genomic data called BeetleBase, making the information more accessible (Kim et al. 2009). What makes *T. castaneum* even more useful as a model organism is its strong RNAi response. If used for gene knock-down, its efficiency is almost 100%. RNAi has been used extensively for the identification of gene functions, including genes involved in the formation of the cuticle (Arakane et al. 2005) and pigmentation (Arakane et al. 2009). Furthermore, a broad screening of RNAi phenotypes has been performed and made available in the iBeetle-Base (Dönitz et al. 2015).



**Figure 6.** Developmental stages of egg, larvae, pupae, and adult of the red flour beetle *Tribolium castaneum*.

The widespread distribution of *T. castaneum* in laboratories around the world led to it becoming the first insect to have its genome sequenced in 2008, using BAC libraries and shot gun Sanger sequencing. Approximately 80% of the whole genomic content was assembled into the 10 linkage groups, which include 9 autosomes and the X chromosome, while the remaining 20% was grouped into scaffolds and singletons (Richards et al. 2008). Genome assembly and the corresponding gene set were further improved in 2020, but the repetitive portion of the genome remained largely unassembled (Herndon et al. 2020).

Under non-laboratory conditions, *T. castaneum* is a cosmopolitan pest reported in 156 countries and is found in dry agricultural storages, primarily of wheat and rice (Table 1). It has been spread by humans throughout the world, mainly through transportation by cargo ships, which has allowed its growth in controlled indoor environments of storehouses even in countries with unsuitable climates. Due to the same characteristics that make it a great model organism, such as longevity and rapid reproductive rates, it spreads through different facilities and causes economic damage through its feeding habits, but also through the dissatisfied consumers of infested goods (Campbell et al. 2021).

### 1.2.2. Other species of the genus *Tribolium*

Apart from *T. castaneum*, the representative of the genus *Tribolium*, seven more *Tribolium* species have been used in various research studies (Table 1). Most of the remaining species have not been mentioned in the literature since they were described as species by Hinton in 1948 (Angelini and Jockusch 2008). These include species of the *alcine* and *myrmecophilum* group, which remain underrepresented in research. The investigated species of *T. castaneum*, *T. freemani*, *T. madens*, *T. audax*, *T. confusum*, *T. anaphe*, *T. destructor* and *T. brevicornis* are divided into the three remaining groups – *castaneum*, *confusum* and *brevicornis* (Table 1).

#### Castaneum group

Within the *castaneum* group, *T. freemani* is most closely related to the representative *T. castaneum*. The native areal of *T. freemani* is considered to be in the Kashmir region on the Indian subcontinent, as the first specimen of the species was found there in 1893 (Table 1). After its discovery, however, the beetle was transferred to the British Museum and was forgotten until it was described as a new species by Hinton in 1948 (Hinton 1948). The next record of *T. freemani* was in 1978 in Japan, where it was discovered as an infestation in a shipment of maize imported from Brazil. Subsequently, these beetles were successfully propagated in laboratory culture. Further studies found that individuals of *T. castaneum* and *T. freemani* can hybridize, and that hybridization produces numerous but sterile offspring (Nakakita et al. 1981), confirming Hinton's morphological observations

of their close relationship as sibling species. Further hybridization experiments confirmed that the two species share homologous genes with very similar genetic content (Brownlee and Sokoloff 1988; Spray and Sokoloff 1995). From an evolutionary standpoint, the hybridization experiments determined that the reproductive isolation of *T. castaneum* and *T. freemani* is postcopulatory and prezygotic. This conclusion was made by the simultaneous copulation of female specimens of *T. castaneum* with the males of both species, in which sperm produced by the conspecific male was strongly preferred in 99% of cases, establishing that the reproductive barrier occurs after copulation and before zygote formation (Robinson et al. 1994; Wade and Johnson 1994). The high-quality reference genome of *T. freemani* Tfree1.0, assembled from sequences generated by PacBio HiFi sequencing with 99.8% completeness of the conserved insect genes, was published in 2022 (Volarić et al. 2022). Using the assembled *T. castaneum* genome as a reference, the *T. freemani* genomic sequence was assembled into 10 linkage groups composed of nine autosomes and the X chromosome. Although the genome size of *T. freemani* was experimentally determined to be 0.238 pg or approximately 230 Mb (Alvarez-Fuster et al. 1991), bioinformatic analyses show that the more accurate number is 320 Mb (Figure 5) (Volarić et al. 2022). Cytogenetically, *T. freemani* also shows karyotypic similarity with *T. castaneum*. Moreover, it has a diploid set of 20 chromosomes with the meiotic formula of  $9 + Xy_p$  (Figure 5), where the  $Xy_p$  refers to the large blocky X chromosome and the corresponding small y chromosome, which together resemble a parachute during metaphase I. The chromosomes of both species vary in their morphology, ranging from acrocentric and submetacentric to metacentric.

The *castaneum* group of the genus *Tribolium* also contains the second pair of sibling species, namely the black flour beetle *T. madens* and the American black flour beetle *T. audax*. The exact country of origin of *T. madens* is unknown, but it was first found in a beehive in Silesia, Poland, and subsequently throughout Europe (Hinton 1948). Due to the very similar phenotype, specimens of *T. madens* and *T. audax* were initially grouped together as a single species *T. madens*. Due to the slight morphological differences in beetles found in a grain elevator in the USA, the taxonomy was revised and *T. audax* was described as a new species. However, the two species are able to hybridize and produce a few sterile progenies, which consolidated their status as sibling species (Halstead 1969). Both species have a diploid number of 20 chromosomes, but with additional smaller supernumerary or B chromosomes with the meioformula of *T. madens* being  $9 + Xy_p + BII\ 3 + BI\ 2$  (Figure 5) (Shimeld 1989).

### *Confusum* group

The African continent is considered to be the area of origin of the beetles in the *confusum* group. The most notable beetle of the group is the confused flour beetle *T. confusum*, but the destructive flour beetle *T. destructor* and flour beetle *T. anaphe* were also studied in laboratories (Table 1). Interestingly, *T. confusum* was found in an Egyptian pharaonic tomb estimated to date back

to 2500 BC (Hinton 1948). It is a globally spread pest in storages of food but also has a long history as a model organism (Pointer et al. 2021). *T. confusum* emerged as a research subject 100 years ago when it was recognized as a good model for nutritional studies (Chapman 1924). In more contemporary laboratories, it has been used in various agricultural studies related to pest management due to its cosmopolitan pest status (Agrafioti et al. 2023; Berhe et al. 2024). Moreover, its close evolutionary relationship with another important pest, *T. castaneum*, makes the two excellent organisms for the study of population dynamics (Holditch and Smith 2020; Cronin et al. 2023).

Even though the typical diploid chromosome number for coleopteran species is 20, the chromosome number of the three species from the *confusum* group mentioned here is  $2n = 18$ , with the meioformula of  $8 + \text{neo-XY}$  (Figure 5) (Smith 1952; Shimeld 1989). It is hypothesized that the reduction in chromosome number was due to the fusion of the sex chromosomes with a pair of autosomes, which are therefore named neo-X and neo-Y. For this reason, neo-X is larger than the other chromosomes of the complement, while neo-Y remains relatively small (Smith 1952).

#### *Brevicornis* group

The *brevicornis* group and its representative, the North American flour beetle *T. brevicornis*, are the most basal *Tribolium* group in evolutionary terms. However, their position within the genus *Tribolium* is controversial, and various evolutionary studies have been published exploring it with contradictory results. The evolutionary study regarding the phylogeny of *Tribolium* beetles was conducted by analyzing the mitochondrial DNA cytochrome oxidase I and 16S RNA as molecular markers determining the monophyly of the genus. The eight species of the three *Tribolium* groups were included - *T. castaneum*, *T. freemani*, *T. madens*, *T. audax* (*castaneum* group), *T. confusum*, *T. anaphe*, *T. destructor* (*confusum* group) and *T. brevicornis* (*brevicornis* group) (Meštrović et al. 2006). These results were largely in accordance with the previous research done by Hinton in 1948, who used morphological characteristics. In another study performed on the same species, using two mitochondrial and three nuclear molecular markers, the authors supported the *castaneum* and *confusum* groups as monophyletic, but questioned the position of *T. brevicornis* within the genus (Angelini and Jockusch 2008). They argued that the species of the *brevicornis* group should be considered part of the genus *Aphanotus*, making *Aphanotus brevicornis* the alternative species name to *T. brevicornis*. In the most recent study, which used improved techniques of Bayesian methods for the estimation of the divergence of species, *T. brevicornis* was found to have split from the rest of the genus around 86 Mya, solidifying its position within the genus *Tribolium* (Figure 5) (Ramesh et al. 2021). Cytogenetically *T. brevicornis* has  $2n = 18$  chromosomes, with a meioformula of  $9 + \text{neo-XY}$  and chromosomes neo-X and neo-Y similar to those of *T. confusum* (Shimeld 1989).

### 1.3. Satellite DNAs of the *Tribolium* species

The species of the genus *Tribolium* are an excellent experimental model for the study of repetitive DNA, especially satDNAs. Using classical molecular biology methods, the major satDNAs have been characterized in genomes of eight species (Table 2), namely *T. castaneum* (Ugarković et al. 1996a), *T. freemani* (Juan et al. 1993), *T. madens* (Ugarković et al. 1996b), *T. audax* (Mravinac and Plohl 2010), *T. confusum* (Plohl et al. 1993), *T. anaphe* (Mravinac et al. 2004), *T. destructor* (Mravinac et al. 2004), and *T. brevicornis* (Mravinac et al. 2005). Curiously, their monomer units show no similarities in nucleotide sequences, with the exception of the major satDNAs of the sibling species pair *T. madens* and *T. audax* (Mravinac and Plohl 2010). However, what they have in common is the high A+T composition of their monomer consensus sequence, with the lowest value being 69% (Table 2). Furthermore, all characterized major satDNAs dominate the (peri)centromeric heterochromatic regions of chromosomes.

**Table 2.** Major satellite DNAs of the eight species of the genus *Tribolium* and their basic characteristics.

species	satDNA	monomer length (bp)	genome proportion%	A+T composition%
<i>T. castaneum</i> <sup>1</sup>	TCAST	360	17	73.1
<i>T. freemani</i> <sup>2</sup>	TFREE	166	31	70.5
<i>T. madens</i> <sup>3</sup>	TMAD1	225	30	74
	TMAD2	704	4	70
<i>T. audax</i> <sup>4</sup>	TAUD1	110	40	72.3
	TAUD2	1412	19.3	70.8
<i>T. confusum</i> <sup>5</sup>	TCONF	158	40	73
<i>T. anaphe</i> <sup>6</sup>	TANAPH	161	13.2	69
<i>T. destructor</i> <sup>6</sup>	TDEST	145	0.7	79.3
<i>T. brevicornis</i> <sup>7</sup>	TBREV	1061	21.2	69.2

<sup>1</sup>Ugarković et al. 1996a, <sup>2</sup>Juan et al. 1993, <sup>3</sup>Ugarković et al. 1996b, <sup>4</sup>Mravinac and Plohl 2010, <sup>5</sup>Plohl et al. 1993, <sup>6</sup>Mravinac et al. 2004, <sup>7</sup>Mravinac et al. 2005

The satellitome of the red flour beetle *T. castaneum* has been studied extensively. The major satDNA TCAST was detected by restriction digestion, and its genomic abundance was determined to be 17% (Ugarković et al. 1996a). Further studies revealed the existence of five different subfamilies of TCAST with their consensus sequence similarity being 70% and a variation in monomer length between 332 and 384 bp due to insertion and deletion events (Felicciello et al. 2011; Pavlek et al. 2015). Differentiation between them could not be achieved by FISH experiments, as all of the variants showed identical localization in the (peri)centromeric region of all 20 chromosomes (Pavlek et al.



2015). Furthermore, TCAST was found to be the dominant centromere-competent DNA sequence, co-localizing with the centromeric histone H3 variant of *T. castaneum*, called cCENH3, with centromeres spreading over 40% of the chromosome length (Gržan et al. 2020). Ten new additional satDNAs of *T. castaneum* were discovered in the first version of the genomic assembly, but their genomic abundance is generally no more than 1%, making them moderate copy (Felicciello et al. 2014; Pavlek et al. 2015). In contrast to the heterochromatic TCAST, the previously mentioned satDNAs are found in euchromatic regions in most cases (Pavlek et al. 2015). The satellitome of *T. castaneum* was expanded with the discovery of 46 new low-copy satDNAs using Illumina reads and the TAREAN pipeline, bringing the total satDNA number in the *T. castaneum* genome to 57 (Gržan et al. 2023). Novel satDNAs together comprise 2.1% of the total genomic content and 45 of them are present in the genome with a proportion of less than 0.01%. As seen with the major satDNAs, the low-copy satDNAs of *T. castaneum* are skewed towards a high A+T composition of more than 60% (Gržan et al. 2023).

Even though *T. freemani* is a sibling species of *T. castaneum*, the nucleotide sequence of the major satDNA found in its genome shows no similarities with the TCAST satDNA. The consensus sequence of the TFREE satDNA is 166 bp long, A+T rich and comprises 31% of the genome (Table 2). Regarding the complexity of the monomer unit, four different inverted repeat regions of 20 bp or less were identified in its sequence, which presumably form cruciform structures (Figure 7A) (Juan et al. 1993). Juan et al. located TFREE in the centromeric chromosome areas and concluded that the satDNA is pericentromeric, but do not mention whether it is present on all of the chromosomes.

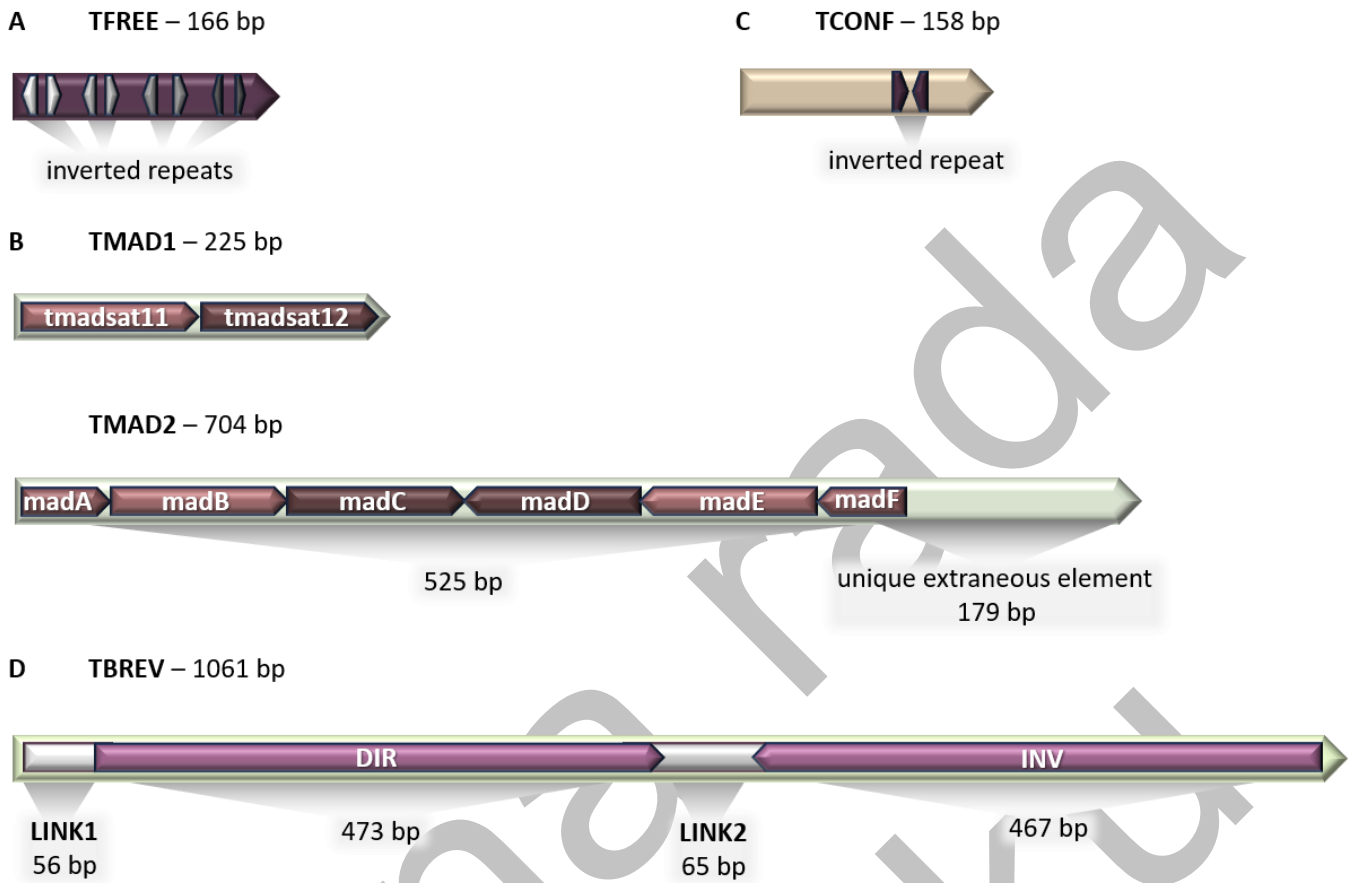
The monomers of the major satDNAs of *T. madens*, TMAD1 and TMAD2, exhibit a complex organization. The monomer of TMAD1 is 225 bp long and consists of two subunits that are derived from the same ancestral subunit and have a pairwise identity of 66.1% (Figure 7B) (Ugarković 1996b). The HOR organization of TMAD2 is even more complex. It has two different subfamilies both of which have evolved by amplification of a 110 bp subunit homologous to the subunits of TMAD1. The 110 bp sequence is repeated two and a half times, followed by an inverted duplication of the same region, which accounts for 525 bp of the 704 bp long monomer. The remainder of the sequence is a unique extraneous element (Figure 7B) (Ugarković 1996b). According to the FISH localization experiments, TMAD1 and TMAD2 are located in large blocks throughout the whole pericentromeric area of every chromosome in the complement, including the supernumerary chromosomes. TMAD2 is primarily interspersed within the longer arrays of TMAD1, with only 15% of the total monomers located in arrays consisting of more than five TMAD2 monomers (Durajlija et al. 2000). Interestingly, the structure, sequence and organization of the two major satDNAs TAUD1 and TAUD2 in the sibling species of *T. audax* closely mirrors those of the major satDNAs of *T. madens* (Mravinac and Plohl 2010). The origin sequence of TAUD1 and TAUD2 is also an approximately 110 bp long fragment, with a 110 bp also being the monomer length of TAUD1 (Table 2). The monomer of TAUD2 is 1412 bp long and consists of 14 amplified 110 bp long fragments, with two inverted segments within the repeat unit,

possibly forming a hairpin formation. By comparing the nucleotide sequences of the major satDNAs of *T. madens* and *T. audax* using maximum parsimony, it was determined that the 110 bp fragments of TMAD1 and TAUD1, as well as those of TMAD2 and TAUD2, are more homologous to each other than the fragments derived from the same species. Furthermore, TAUD2 is interspersed within the arrays of TAUD1, as is the case for TMAD1 and TMAD2 (Mravinac and Plohl 2010).

The major satDNAs of the three species of the *confusum* group, *T. confusum*, *T. anaphe* and *T. destructor*, do not share significant homologies with other major satDNAs in the nucleotide sequences of their monomer unit. Experimental data suggests that TCONF of *T. confusum* is the most abundant of all, comprising 40% of the genome (Table 2). Its monomer is 158 bp long (Table 2) and consists of several A+T rich direct repeats with one 28 bp long inverted repeat that could possibly make a cruciform structure (Figure 7C) (Plohl et al. 1993). Both TANAPH and TDEST are also A+T rich with inverted repeats (Table 2). However, the low genomic abundance of TDEST is the outlier in the genus, but TDEST is still distributed in large heterochromatic blocks as shown by FISH experiments (Mravinac et al. 2004).

TBREV, the major satDNA of *T. brevicornis*, has a 1061 bp long and complex HOR monomer unit. It is composed of two large inverted subunits called DIR and INV, which are 473 bp and 467 bp long, respectively, and only 18% diverged from each other. For this reason, the monomer has a strong potential of forming the stable dyad structure. The subunits are divided by two unique linker segments, LINK1 and LINK 2 (Figure 7D) (Mravinac et al. 2005). Although the known major satDNAs of species in the genus *Tribolium* lack homology in their sequences, some of their similarities, such as high A+T composition and small inverted repeats, suggest that the structure of the pericentromeric regions is more important for chromatin formation than the conserved sequences (Mravinac et al. 2005).

The low-copy satDNAs of the *Tribolium* species, except for the representative *T. castaneum*, have yet to be described. Therefore, their satellitomes have been unknown until the research done in this dissertation.



**Figure 7.** Schematic representation of structure and organization of monomers of *Tribolium* major satellite DNAs TFREE (A), TMAD1 and TMAD2 (B), TCONF (C) and TBREV (D).

#### 1.4. Aim and hypotheses of the study

The aim of this study is to define and comprehensively study the satellitomes of four insect species of the genus *Tribolium*, namely *T. freemani*, *T. madens*, *T. confusum* and *T. brevicornis*, by combining bioinformatics and experimental approaches. The main purpose of the analysis of each satellitome is to determine the composition of the satellite collections and genomic organization of satDNAs within each of the species. Ultimately, the comparison of the satellite profiles will determine how the satDNAs evolved and possibly clarify how the evolution of satDNA has influenced the shaping of their genomes and speciation.

Major satDNAs of the four *Tribolium* species have already been characterized, but their potential low-copy satDNAs remain unexplored. The preliminary hypothesis of this study is that the genomes of *T. freemani*, *T. madens*, *T. confusum* and *T. brevicornis* contain multiple different uncharacterized satDNA families. Because of the fact that their major satDNAs do not share sequence similarities, it can be hypothesized that some of their low-copy satDNAs will also be species-specific. Furthermore, some of the satDNAs could be shared among multiple analyzed species, evolving following the concept of concerted evolution.

## 2. METHODS AND MATERIALS

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## 2.1. Materials

### 2.1.1. Model organisms

In this research, insects of the species *Tribolium freemani*, *Tribolium madens*, *Tribolium confusum* and *Tribolium brevicornis* were used. The initial cultures were obtained from the Insectarium of the Agricultural Research Service (Manhattan, Kansas, USA) at the United States Department of Agriculture. The cultures were continuously propagated in standard laboratory conditions (27°C, 50-75% humidity, 75% whole wheat flour, 25% barley/rye flour with addition of barley/rye) with a change of flour every 2 – 4 weeks.

### 2.1.2. Chemicals

#### Chemicals used in this research:

- ethidium bromide (Promega)
- ethanol, NaOH, acetic acid, KCl, NaCl, Na-citrate, Na-acetate, Na<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub> (Kemika)
- agarose LE, orange G, Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), Tris, formaldehyde, blocking reagent, Tween20, maleic acid, colcemide (Sigma-Aldrich)
- 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal), isopropyl-beta-D-thiogalactopyranoside (IPTG) (Gibco-BRL)
- formamide (Merck)

### 2.1.3. Buffers and solutions

#### Buffers and solutions used in this research:

- 20x SSC buffer (pH 7.0): 3M NaCl, 0,3 M Na-citrate
- 10x PBS buffer (pH 7.4): 137 mM NaCl, 2.7 mM KCl, 10.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>
- 50x TAE buffer (pH 8.0): 2 M Tris, 1 M acetic acid, 50 mM EDTA
- 5x TBE buffer: 446 mM Tris base, 445 mM boric acid, 10 mM EDTA (pH 8.0)
- TE buffer: 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)
- liquid medium for bacterial growth LB (pH 7.0): Bacto Tryptone (10 g/L), yeast extract (5 g/L), NaCl (10 g/L)
- liquid medium SOC: 2% Bacto Tryptone, 0,5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl<sub>2</sub>, 10 mM MgSO<sub>4</sub>, 20 mM glucose (Merck)

- solid medium for bacterial growth LB with antibiotic (pH 7.0): LB agar (40 g/L) and ampicillin (0.1 µg/L)
- buffer G (genomic DNA isolation buffer): 0.1 M NaCl, 0.01 M Tris-HCl (pH 8.0), 25 mM EDTA (pH 8.0), 0,5% SDS
- phenol:chlorophorm:isoamyl alcohol mixture (25:24.1) (Merck)

Labeled nucleotides and commercial antibodies:

- Biotin-16-(5-aminoallyl)-dUTP, Aminoallyl-dUTP-Cy3 (Jenna Bioscience)
- Biotinylated Goat Anti-Avidin D Antibody, Streptavidin Fluorescein (Vector Laboratories)

Buffers and solutions for FISH experiments:

- denaturation solution: 70% formamide in 2x SSC buffer
- buffer DeSO<sub>4</sub> (pH 7.0): 4x SSC, 20% dextran sulphate, 50 mM Na-phosphate
- hybridization buffer: 60% deionized formamide, 40% DeSO<sub>4</sub> buffer
- washing buffer: 50% formamide in 2x SSC buffer
- 4M buffer: 4x SSC, 5% blocking reagent
- 4T buffer: 4x SSC, 0.05% Tween20
- lysis buffer for chromatin fiber slide preparation: 200 mM Tris-HCl, pH 7.4, 50 mM EDTA, 0.2% SDS, 1 mM PMSF
- fixative (acetic acid:ethanol = 1:3)
- 45% acetic acid solution
- DAPI solution in 2xSSC: 50 ng/ml, 2x SSC
- 70%, 90% and 100% cold ethanol
- 2x SSC in 50% formamide
- *antifade* reagent Mowiol 4-88 (Sigma-Aldrich)
- phenylmethylsulfonyl fluoride (PMSF) (Merck)
- Image-iT Signal Enhancer (Cell Signalling Technologies)

Buffers and solutions for Southern blot hybridization:

- hybridization buffer: 0,25 M phosphate buffer (pH 7.2), 1 mM EDTA (pH 8.0), 20% SDS, 0.5% blocking reagent
- washing buffer: 20 mM Na<sub>2</sub>HPO<sub>4</sub>, 1 mM EDTA, 1% SDS
- buffer 1 (pH 8.0): 0.1 M maleic acid, 3 M NaCl, 0.3% Tween20

- buffer 2: buffer 1, 1% blocking reagent
- buffer 3: 0.1 M Tris-HCl (pH 9.5), 0.1 M NaCl
- stripping buffer: 0.2 M NaOH, 0.1% SDS
- chemiluminescent substrate CDP-star (1:50 in buffer 3)

#### **2.1.4. Commercial kits**

##### Commercial kits used in this work:

- "E.Z.N.A. Insect DNA Kit" for isolation of high molecular weight insect DNA (Omega Bio-tek)
- "QIAquick PCR Purification Kit" for purification of PCR reactions (QIAGEN)
- "Monarch® PCR & DNA Cleanup Kit (5 µg)" for purification of PCR reactions (New England Biolabs)
- "QIAquick Gel Extraction Kit" for purification of DNA fragments from agarose gel (QIAGEN)
- "GoTaq Flexi DNA Polymerase" for polymerase chain reaction, which contains: GoTaq DNA polymerase (5U/µl), 5xGoTaq Flexi concentrated buffer and 25 mM MgCl<sub>2</sub> (Promega)
- "GoTaq Green Master Mix" kit for polymerase chain reaction, with 2x Green GoTaq reaction buffer which contains: GoTaq DNA polymerase, 400 µM dNTP mixture and 3 mM MgCl<sub>2</sub> (Promega)
- "pGEM-T Easy Vector System I" for cloning PCR products, which contains: plasmid vector pGEM-T (50 ng/µl), enzyme T4 DNA ligase (3 Weiss U/µl) and 2x concentrated buffer for T4 DNA ligase (Promega)
- "High Pure Plasmid Isolation Kit" for plasmid isolation (Roche Applied Science)
- "Nick Translation Mix" for fluorescent labelling (Roche)

#### **2.1.5. Enzymes**

- Exonuclease V (RecBCD) with corresponding buffers (New England Biolabs)
- RNase A, proteinase K (Thermo Scientific)
- pepsin powder (Sigma-Aldrich)

#### **2.1.6. Markers and ladders for gel electrophoresis**

- GeneRuler DNA Ladder Mix, ready-to-use containing 21 DNA fragments in size range from 100 bp to 10000 bp (Thermo Scientific)



- Quick-Load® 1 kb Extend DNA Ladder, size range 0.5 kb to 48.5 kb (New England Biolabs)

### 2.1.7. Plasmid vector

The plasmid vector pGEM-T Easy from the kit "pGEM-T Easy Vector System I", which enables AT cloning, was used to clone the PCR products. The Taq polymerase adds dATP to the 3' ends of the PCR product, which is paired with complementary thymidine at the ends of the linear T vector in the ligation reaction.

The vector pGEM-T Easy contains a gene for resistance to the antibiotic ampicillin (apm<sup>r</sup>), which enables selection of the transformed bacteria. The site for fragment cloning is located within the lacZ gene, which codes for the enzyme  $\beta$ -galactosidase, so that blue-white selection of the transformants is possible by  $\alpha$ -complementation.

### 2.1.8. Bacterial strain

The bacterial strain XL10-Gold of *Escherichia coli* (Tet<sup>r</sup> $\Delta$ (mcrA)183  $\Delta$ (mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA 1gyrA96 relA1 lac Hte [F' proAB lac<sup>q</sup>Z $\Delta$ M15 Tn10 (Tet<sup>r</sup>) Amy Cam<sup>r</sup>] (Agilent) were used for plasmid propagation.

### 2.1.9. Technical equipment and machines

Technical equipment and devices used in this work:

- PCR 2720 Thermal Cycler (Applied Biosystems)
- device for horizontal DNA electrophoresis of agarose gels (Bio-Rad)
- UV transilluminator with system for recording agarose gels G:BOX (Syngene)
- hybridization oven OV1 (Biometra)
- table microcentrifuge Minispin (Eppendorf)
- table shaker Vibramax (Heidolph)
- thermoblock (Bioblock Scientific)
- ThermoMixer C with ThermoTop (Eppendorf)
- device for quick drying of samples Speed vac DNA120 (Thermo)

- dryer (Shel Lab)
- thermostatic incubator (Sutjeska)
- water bath (Inko)
- device for sealing of plastic bags (Gorenje)
- photographic red light (Kaiser)
- device for fluorometric measurement of DNA concentration Qubit 4 (Invitrogen)
- confocal laser scanning microscope Leica TCS SP8 X (Leica Microsystems)
- Cytospin 4 (Thermo Scientific)
- SteREO Discovery.V20 stereo microscope (Zeiss)
- chemiluminescence and spectral fluorescence imaging system Alliance Q9 mini (Uvitec)

## 2.2. Methods

### 2.2.1. Genomic DNA isolation

The total genomic DNA of the *Tribolium* species was isolated from insect tissue using organic solvents according to the protocol for the isolation of genomic DNA. The insect tissue was frozen in liquid nitrogen and crushed with a mortar and pestle. The crushed tissue was resuspended in buffer for the isolation of genomic DNA (100 mg tissue in 1 ml buffer) with the addition of proteinase K (0.25 mg/ml) and incubated in a water bath at 50 °C for approx. 18 hours. An equal volume of phenol (pH 8.0) was added to the suspension. The solution was mixed by swirling and centrifuged at 12,000 rpm for 9 minutes. After centrifugation, the aqueous phase was transferred to a new microtube and the procedure was repeated twice. In the next repetition, an equal volume of phenol:chloroform:isoamyl alcohol solution in the ratio 25:24:1 was added to the aqueous phase, whereupon the genomic DNA was found in the upper aqueous phase. The genomic DNA was precipitated with 0.1 initial volume of 3M sodium acetate (pH 4.7) and two initial volumes of ice-cold 100% ethanol by incubation at room temperature for 10 minutes. After incubation, the solution was centrifuged for 15 minutes at a rotation speed of 12,000 rpm. The supernatant was removed, and 75% ice-cold ethanol was added to the precipitate. The solution was centrifuged at 12,000 rpm for 7 minutes. The supernatant was carefully removed with a vacuum pump and the precipitate was dried at room temperature (~30 min). After the precipitate was completely dried, it was dissolved in 1× TE buffer (pH 8.0). RNase A (10 µg/ml) and SDS (0.1%) were added to the solution and incubated for 1 h at 37 °C in a water bath. Extraction was performed with a phenol:chloroform:isoamyl alcohol solution. The sample was precipitated in 0.1 volumes of 3M sodium acetate (pH 4.7) and 2 volumes of ice-cold 100% ethanol by incubation at room temperature for 10 minutes followed by centrifugation at 12,000 rpm for 15 minutes. The precipitate was washed with 75% ice-cold ethanol and the solution was centrifuged at 12,000 rpm for 7 minutes. After centrifugation, the supernatant was removed, and the precipitate was dried completely at room temperature. The dry residue was dissolved in 1×TE buffer (pH 8.0). Quantity and quality of isolated total genomic DNA were assessed by electrophoresis in a 1% agarose gel by comparison with DNA of known concentration. The concentration was also confirmed by fluorometric measurement using the Qubit 4 instrument.

High molecular weight DNA (HMW DNA) was isolated using the “E.Z.N.A. Insect DNA Kit” by modified protocol published by Oppert et al. (2019) from 25 mg of pupal or larval tissue and by protocol described by Volarić et al. (2021) from 1 g of pupal tissue. The concentration was measured using the Qubit 4 fluorometer.

## 2.2.2. Polymerase chain reaction (PCR) amplification of DNA fragments

The DNA segments of potential satellites were amplified using the PCR.

### 2.2.2.1. PCR primers

PCR primers were used to amplify segments of different potential satDNAs. The nucleotide sequences of the primers and their annealing temperatures are listed in Table 3. The listed oligonucleotide primers were synthesized at the Macrogen Europe (Amsterdam, The Netherlands) customer service. Gradient PCR was used to determine the optimal pairing temperatures of each primer pair with the DNA template.

**Table 3.** Nucleotide sequences and annealing temperatures of PCR primers used in this study.

satDNA	primer sequence	Ta °C	satDNA	primer sequence	Ta °C
TfrSat01	5'-CAAGGGTCGAAACAGTTCCA-3'	56	TmaSat09	5'-TATCCGCATTACACCTTTATCT-3'	57
	5'-CAGGCAATCGATTGAAGTTCAAAA-3'			5'-GGTAAACTGCCAATACATTACGC-3'	
TfrSat03	5'-AACGTTTGCTGGTTTGAAG-3'	54	TmaSat10	5'-ACTTTACAGTCTGATCATAGCCT-3'	58
	5'-ACAAAAACGGCAAATTCAGAC-3'			5'-TCTACTGAAAACAGAGAGCAAT-3'	
TfrSat04	5'-AAGACGTTTTAGTGAGTTTTATG-3'	65	TcoSat01	5'-TTTTACTGGTGTATACGTAGGTC-3'	55
	5'-GATTTTTGTACAGTTGGATGAA-3'			5'-ATTGCTTTATCTCTACTAATAGCTG-3'	
TfrSat05	5'-GGCGCATCCACCATTTTTCA-3'	62	TcoSat02	5'-TCCTACTTTTATAACGTTTCAGCA-3'	59
	5'-TGTTCCATTGAATTCTGCGG-3'			5'-TCTGCTCAATCGAATGGT-3'	
TfrSat06	5'-TGCCTAATCAGATTCTCTTGCAA-3'	57	TcoSat03	5'-CTATTGTTTTTATACTTTTGCA-3'	53
	5'-TGGCATATCTCAATTTGTTTTGTCAG-3'			5'-CAATCTACATTCTCCAGCTTGT-3'	
TfrSat07	5'-TTTCCACCCTGTTCCAAA-3'	55	TcoSat04	5'-AACGTTTACTATCAGGCTT-3'	56
	5'-AAAATTCATTAAGGTCCCAAGGG-3'			5'-AAGAGAATGAGATTTGGGACA-3'	
TfrSat09	5'-CGGTGTTCCCAAGCAAATT-3'	51	TcoSat05	5'-CAATATGGAGGAATAAAATATAA-3'	50
	5'-TTTGCACTTACATTCTAAATATTCT-3'			5'-AACATATAATTATCTCGAAACTT-3'	
TfrSat10	5'-GTTTTTCAACGATTCAAGTACT-3'	59	TcoSat06	5'-AGAATGTTGTCTGACCTGCT-3'	59
	5'-AAGAGTGAACCTTCCG-3'			5'-TGGCTAAATCACCGTGGATT-3'	
TfrSat11	5'-CCCTAATTTGAATATGTCAACTCTG-3'	61	TcoSat07	5'-TCTACAAGTGAAAGAACGACG-3'	59
	5'-TTTTCTCTACCCTACCCTT-3'			5'-GTGGAAGACTGGTTGGAG-3'	
TmaSat01	5'-TCCGATTTGGGTCAATT-3'	55	TcoSat08	5'-AAAATAATTCGCTCCCCTCGA-3'	59
	5'-TCAAAAATACACAATTGCTT-3'			5'-GAAATACCATTTACACAGTTGA-3'	
TmaSat04	5'-CCCGAAAAACCCTCTGAGAT-3'	56	TcoSat09	5'-AGGAGAAGGATAAGTCACCAGA-3'	59
	5'-TGTAGGAGTTTTGTCTTAGGAGT-3'			5'-AGGTGTCAATTAATTTAGTAGGT-3'	
TmaSat05	5'-AGCTTAAGGACTGATGGCCG-3'	56	TbrSat02	5'-GACCAAGGATTTAAATTCATAA-3'	54
	5'-AGCGCTTAAAATCGTTGAAAAAC-3'			5'-CAATTTGGATGTTTGTGAAGT-3'	

<b>TmaSat06</b>	5'-GGTTACAATGGCTTTTTAGGGCT-3'	56	<b>TbrSat03</b>	5'-AAAACTGTCTTTGTGTAAAAA-3'	50
	5'-CGATACACGGGATGACACTTGA-3'			5'-ACTAGTATCCGTAGCTTAAAAA-3'	
<b>TmaSat07</b>	5'-TCGTAGTATTTAAAACCGGAACCC-3'	56	<b>TbrSat05</b>	5'-GGTACAAATCTTCCACTAAT-3'	50
	5'-GCTAAAAGCGATATTACAGGTCTCT-3'			5'-TGTCTGGGGCCAAAACATT-3'	
<b>TmaSat08</b>	5'-GGTAAACCGACATCAGCCAA-3'	58	<b>TbrSat10</b>	5'-CTGACCTCCCAAATTTTGCC-3'	50
	5'-GGGCGTTTTGGGAGATTTG-3'			5'-GATGGCCGAATGAGAAACCT-3'	

#### 2.2.2.2. PCR reaction mixtures and conditions

The reaction mixtures for the amplification of satDNA using the PCR method were prepared in a total volume of 30  $\mu$ l. The mixture contained 5 $\times$ GoTaq Flexi Buffer, 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTP mix, 0.4  $\mu$ M specific primers, 0.25U GoTaq polymerase and 10 ng genomic DNA. The PCR program for amplification of satDNAs is listed in Table 4. Specific annealing temperatures for satDNAs can be found in Table 3.

**Table 4.** PCR program for amplification of satellite DNAs. Specific annealing temperatures (marked with an asterisk) can be found in Table 3.

	Temperature	Time	PCR cycles
Initial denaturation	94 °C	3 min	1
Denaturation	94 °C	10 s	35
Annealing*	50 – 65 °C	10 s	35
Extension	72 °C	10 s	35
Final extension	72 °C	5 min	1

#### 2.2.3. Agarose gel electrophoresis for separation of DNA

The DNA fragments amplified by the PCR were separated by electrophoresis in a 1% agarose gel. The gel was prepared by dissolving agarose in 1 $\times$ TAE buffer and adding ethidium bromide (0.5  $\mu$ g/ml) to visualize the DNA. Electrophoresis was performed at room temperature in the same buffer.  $\lambda$ -DNA of known concentration was used to estimate the amount of DNA, and the "Quick Load 2-Log DNA Ladder" electrophoresis marker was used to estimate the length of the segments. After electrophoretic separation, the gels were photographed with the G:BOX device.

#### **2.2.4. Extraction of DNA from agarose gels**

Parts of the agarose gel with the desired DNA segment were cut on a transilluminator and weighed. The DNA was isolated using the "QIAquick Gel Extraction Kit" according to the manufacturer's instructions.

#### **2.2.5. Cloning of DNA fragments**

The DNA segments were cloned using the pGEM-T plasmid vector from the "pGEM-T Easy Vector System I" kit, which enables AT cloning.

##### *2.2.5.1. Ligation of DNA fragments and plasmid vector*

Ligation reactions of the isolated DNA fragments and the pGEM-T Easy vector were performed using the T4 DNA ligase enzyme in 2×rapid ligation buffer overnight at 4 °C. The ratio of DNA fragments and vector in the reaction was 3:1.

##### *2.2.5.2. Transformation of competent bacterial cells*

Competent *E. coli* bacterial cells were transformed with the pGEM-T Easy vector by the heat shock method. 1 µl β-mercaptoethanol was added to 25 µl of cell solution and the suspension was incubated on ice for 10 minutes. 2 µl of the ligation mixture was added to the prepared ultracompetent cells, followed by incubation on ice for 30 minutes. The cells were subjected to a heat shock of 42 °C for 30 seconds by incubating the cell solution and the ligation mixture in a water bath. The cells were then incubated on ice for 2 minutes and regenerated for 90 minutes in 250 µl pre-warmed SOC medium with continuous shaking at 250 rpm at a temperature of 37 °C. Cells were grown on selective solid LB media with the addition of ampicillin (100 µl/ml), 40 µl inductor IPTG (100 mM) and 40 µl substrate X-gal (20 mg/ml) and incubated overnight at 37 °C.

##### *2.2.5.3. Colony PCR*

The use of the plasmid vector pGEM-T Easy enabled the blue-white selection of the colonies. To determine the size of the embedded DNA segments, PCR was performed on bacterial colonies (colony PCR). A specific bacterial colony was touched with a sterile pipette tip, the tip was immersed in a microtube containing 10 µl mQ-H<sub>2</sub>O and resuspended. The sample was denatured in the PCR instrument at 94 °C for 10 minutes, then cooled on ice and briefly centrifuged. The PCR reaction was

performed in 5  $\mu$ l of a reaction mixture containing 2.5  $\mu$ l 2 $\times$  GoTaq Green Master Mix, 0.5  $\mu$ l denatured bacterial colony, 0.4  $\mu$ M primer M13R-pUC (5'-CAGGAAACAGCTATGAC-3'), 0.4  $\mu$ M primer M13F (5'-GTAAAACGACGGCCAGT-3') and 1.6  $\mu$ l mQ-H<sub>2</sub>O according to the program listed in Table 5.

**Table 5.** PCR program for amplification of bacterial colonies.

	Temperature	Time	PCR cycles
Initial denaturation	94 °C	2 min	1
Denaturation	94 °C	15 s	30
Annealing	54 °C	15 s	30
Extension	72 °C	30 s	30
Final extension	72 °C	5 min	1

The products of PCR amplification of the bacterial colonies were analyzed in a 1% agarose gel. Colonies that contained DNA segments of the expected length were grown in 4 ml of liquid selective medium LB supplemented with ampicillin (100  $\mu$ g/ml) overnight at a temperature of 37 °C and continuous rotation at 225 rpm.

#### 2.2.5.4. Isolation of plasmid DNA

Bacteria grown overnight in the liquid selective medium LB were pelleted by brief centrifugation. The plasmid DNA was isolated using the "High Pure Plasmid Isolation Kit" according to the manufacturer's instructions and eluted from the column with 100  $\mu$ l elution buffer. The concentration and quality of the isolated plasmid DNA was assessed by gel electrophoresis in 1% agarose gel using  $\lambda$ -DNA of known concentration.

#### 2.2.5.5. Sanger sequencing of plasmid DNA and bioinformatical analysis of the cloned fragments

The nucleotide sequences of the cloned DNA fragments were determined by the sequencing service Macrogen Europe (Amsterdam, Netherlands) using the above-mentioned plasmid primer M13R-pUC. The nucleotide sequence of sequenced plasmids was evaluated using Geneious Prime 2023.2.1 (Biomatters Ltd).

### 2.2.6. Labelling of DNA hybridization probes

The hybridization probes were labeled with biotin or cyanine3 by the PCR method or using nick translation. The clones most representative of the consensus sequences of satDNAs were used as a reaction template using a mixture of certain clones specific for a particular satellite DNA.

PCR labelling of hybridization probes of potential satellite DNAs was performed in 25  $\mu$ l of the reaction mixture containing 2.5 mM MgCl<sub>2</sub>, 0.5 mM dATP, 0.5 mM dGTP, 0.5 mM dCTP, 0.32 mM dTTP, 0.18 mM biotin-16-dUTP, 5 U GoTaq DNA polymerase, 0.1 ng template DNA and 10  $\mu$ M specific primers for biotin-labeled probes and 5xGoTaq Colorless buffer, 2.5 mM MgCl<sub>2</sub>, 0.5 mM dATP, 0.5 mM dGTP, 0.5 mM dCTP, 0.3 mM dTTP, 0.2 mM Cy3-dUTP, 5 U GoTaq DNA polymerase, 0.1 ng template DNA and 10  $\mu$ M specific primers for Cy3-labeled probes. The amplification program started with a two-minute initial denaturation at 94 °C and continued with 40 cycles alternating three steps: denaturation at 94 °C for 15 seconds, primer annealing for 15 seconds and DNA synthesis reaction at 72 °C for 30 seconds. The pairing reaction was carried out at the optimal pairing temperatures of each satellite, as indicated in Table 3.

Nick translation labelling was performed for a hybridization probe specific for TbrSat01 satDNA. The reaction mixture contained 0.5 mM dATP, 0.5 mM dGTP, 0.5 mM dCTP, 0.3 mM dTTP, 0.2 mM Cy3-dUTP, nick translation mix and 1  $\mu$ g template DNA, according to manufacturer recommendations. The reaction mixture was incubated for 95 min at 15 °C then stopped by adding 1  $\mu$ l of 0.5 M EDTA and heating to 65 °C for 10 min.

Concentration and quality of labelled probes was checked using 1% agarose gel electrophoresis and Qubit 4 fluorometer.

### 2.2.7. Fluorescence *in situ* hybridization (FISH)

#### 2.2.7.1. Metaphase chromosome slide preparation

The tissue for the preparation of metaphase chromosome slides was isolated from the gonads of male or female *Tribolium* beetles at the pupal stage. The isolated tissue was incubated in colcemid (10  $\mu$ g/ml) for 1 hour. A hypotonic shock condition was achieved by incubation in 0.075 M KCl for 15 minutes, which caused the cells to rupture. The tissue was transferred to a fixative (acetic acid:ethanol in a 1:3 ratio) and incubated for 15 minutes. Slides were prepared using the so-called "squash" technique in 45% acetic acid and then immersed in liquid nitrogen. The slides were completely air-dried and stored at -20 °C until they were used in the FISH experiment.



### 2.2.7.2. Chromatin fibers slide preparation

The preparation of slides with elongated chromatin fibers began with the isolation of male gonads from pupae of the species *T. confusum* in sterile 1xPBS supplemented with 1 mM PMSF. The buffer containing the tissues was changed and 100 µl of fresh buffer was added. The tissue was homogenized using a microtube homogenizer with a sterile tip for 30 seconds. As the volume of the suspension per cytoslide should be 400 µl, the volumes of 1xPBS with 1 mM PMSF were calculated to match final volume of suspension. The cell suspension was then passed through a cell mesh with pores of 100 µm in size.

Subsequently, 400 µl of the cell suspension was distributed in each cell funnel attached to the slides. The suspensions were centrifuged in a Cytospin 4 cytocentrifuge at a speed of 1200 rpm for 10 minutes. The slides were briefly airdried, then 15 µl of lysis buffer was added to each slide and 18 x 18 mm coverslips were placed upon them with an incubation period of 4-10 minutes at room temperature. After completion of the lysis reaction, the coverslip was removed with a knife and then fixed with freshly prepared 2% formaldehyde dissolved in 1xPBS buffer for 10 minutes at room temperature. The fixative was washed three times for 5 minutes each in 1xPBS. Before the prehybridization, slides were rinsed in 45% acetic acid for 10 min at RT.

### 2.2.7.3. Prehybridization and hybridization of the cytogenetic slides

The slides were washed in 2xSSC buffer at 37 °C for 5 minutes, followed by incubation in fresh 2xSSC buffer with RNase A (100 µg/ml) and incubated at 37 °C for 1 hour. After washing the slides in 2xSSC solution for 3x5 minutes at 37 °C, they were treated with 10 mM HCl solution containing dissolved pepsin (100 µl/ml) for 10 minutes at 37 °C. Furthermore, they were washed 2x5 minutes at room temperature in 1xPBS solution and then for 5 min in 1xPBS solution with dissolved 50 mM MgCl<sub>2</sub>. The slides were incubated in a formaldehyde solution (2.7 ml formaldehyde in 100 ml PBS buffer with the addition of 50 mM MgCl<sub>2</sub>) for 10 min, washed in a solution of 1xPBS and dehydrated by passing through a series of cold ethanol (70% → 90% → 100%, 3 min each) and then completely air-dried. Prior to hybridization, the double-stranded chains of the DNA probe and the DNA fragments on the slides were separated by denaturation. The chromosome slides were heated for several minutes at 50-60 °C on the top of a water bath and denatured in a denaturing solution (70% formamide in 2xSSC) for exactly 1:30 min at 70 °C, followed immediately by a series of cold ethanol (70% → 90% → 100%, 3 min each) and dried completely at room temperature. Labelled DNA probes (200 ng) were lyophilized, then 15 µl of hybridization solution (60% formamide, 40% DeSO<sub>4</sub> buffer) was added and the mixture was denatured at 75 °C for 5 min in a water bath. After denaturation, the probes were cooled on ice for a few minutes and applied to the chromosome slides. Hybridization took place overnight in a humid chamber at 37 °C. After hybridization, washes were performed in pre-warmed

2×SSC buffer containing 50% formamide for 4×5 min at 37 °C, followed by 2×SSC buffer washes for 3×5 min at 37 °C.

#### 2.2.7.4. Immunodetection of labelled DNA probes

The immunodetection begins with the incubation of the slides in buffer 4M in a humid chamber at 37 °C for 30 minutes. Under the same conditions, the slides were incubated in buffer 4M with avidin-FITC at a dilution of 1:500. The slides were washed for 3×5 minutes in buffer 4T at room temperature and then incubated for 20 minutes in buffer 4M with  $\alpha$ -avidin-biotin in a ratio of 1:100 inside a humid chamber for signal amplification. Next, the slides were washed in buffer 4T for 3×5 minutes, then 4M buffer containing diluted avidin-FITC at a ratio of 1:2000 was added to the slides, and incubation was carried out for 20 minutes in a humid chamber. The slides were washed 3×5 min in buffer 4T and 5 min in 1×PBS solution and subsequently dehydrated by passing through a series of cold ethanol (70% → 90% → 100%, 3 min each). The slides were stained by incubation in a DAPI solution (50 ng DAPI/ml 2×SSC) for 20 min at room temperature. Excess dye was removed by washing the slides with running water. The slides were further washed with distilled water and allowed to air dry completely. Before microscopy, the slides were embedded in the so-called antifade reagent (Mowiol 4-88).

#### 2.2.7.5. Confocal imaging of cytogenetic slides

The cytogenetic slides were analyzed using a Leica TCS SP8 X confocal laser scanning microscope. The resulting images were processed and analyzed using Adobe Photoshop, V.21.0.3 and the image analyzing program ImageJ2 (Rueden et al. 2017) with the image processing package Fiji (Schindelin et al. 2012) and QuickFigures plugin (Mazo 2021).

### 2.2.8. Two-dimensional (2D) agarose gel electrophoresis for detection of extrachromosomal circular DNA

#### 2.2.8.1. Modification of DNA with Exonuclease V

To preserve circular eccDNAs and eliminate excess linear DNA for the detection of eccDNAs, genomic DNA of *T. freemani* first had to be modified by exonuclease V (ExoV). First, to shear linear DNA, genomic DNA was passed 25 times through a 0.33 mm hypodermic needle. Then, the reaction with ExoV was set up in the following ratio: 1x NEBuffer 4, 1 mM ATP, 10U ExoV and 1  $\mu$ l shared DNA, scaled to initial DNA mass of 20  $\mu$ g. The reaction mixture was incubated overnight at 37 °C, and

stopped by adding EDTA to the concentration of 11 mM in addition to heat inactivation at 70 °C for 30 min. The samples were purified using Monarch® PCR & DNA Cleanup Kit (NEB) following the manufacturer's instructions.

#### *2.2.8.2. 2D agarose gel electrophoresis*

The DNA modified by ExoV was used to perform 2D agarose gel electrophoresis. The first electrophoresis was run in 0.7% agarose prepared in 1xTBE buffer at 0.7 V/cm for 18h without added ethidium bromide (EtBr) to ensure proper separation of fragments. Therefore, the agarose gel was later stained with 1xTBE buffer containing 0.2 µg/ml EtBr. The portion of the gel containing the separated DNA was excised and positioned at a 90° angle to the first electrophoresis. The 1.5% agarose in 1xTBE containing 0.2 µg/ml EtBr was poured around it. The second electrophoresis was run for 3:15 h at 4 V/cm.

### **2.2.9. Southern blot hybridization**

#### *2.2.9.1. Transfer of DNA fragments to the nylon membrane*

After 2D electrophoretic separation, the gel was rinsed in 0.25 M HCl for 30 min to depurinate the DNA fragments and in 0.4 M NaOH for 30 min to neutralize it. A positively charged nylon membrane (Roche Applied Science) was placed on the gel. The DNA fragments were transferred to the membrane by alkaline transfer in 0.4 M NaOH solution overnight. The membrane was neutralized by washing in 2xSSC buffer and completely air dried. The dried membrane was placed in a sterilizer for 20 minutes at 120 °C for further fixation of the DNA fragments.

#### *2.2.9.2. Prehybridization and hybridization of membranes*

Prehybridization of the nylon membrane containing DNA fragments was performed for 2 hours at a temperature of 65 °C in hybridization cylinders containing preheated hybridization solution. Hybridization was then performed in a fresh, pre-warmed hybridization buffer with DNA biotin-labelled probe specific for each satellite added with a concentration of 20 ng/ml hybridization buffer. Hybridization was carried out overnight at a temperature of 65 °C.

### 2.2.9.3. Detection of hybridization signal

After hybridization, the membranes were washed for 3×20 minutes in the wash buffer at a temperature 3°C lower than the hybridization temperature in the hybridization cylinders. The hybridization signal detection steps were performed in plastic tubs on a shaker at room temperature. The membranes were incubated in buffer 1 for 2 minutes and then in buffer 2 for 30 minutes. The next incubation lasted 30 min and was performed in buffer 2 to which the antibody solution was added. A streptavidin-AP conjugate (Roche Applied Science) at a ratio of 1:5,000 was used for the detection of biotin-labelled probes. The membranes were washed 5×8 min in buffer 1 and then 2×3 min in buffer 3. The next incubation step was performed in a dark chamber. The membranes were incubated in a sealed transparent plastic bag in buffer 3, to which alkaline phosphatase substrate CDP-Star (Roche Applied Science) was previously added at a ratio of 1:50. The membranes were visualized by Alliance Q9 mini (Uvitec) and images were processed by Adobe Photoshop, V.21.0.3.

### 2.2.10. Whole genome sequencing

The genomes of the *Tribolium* species were sequenced using two different sequencing platforms by commercial services. Illumina sequencing was performed by Admera Health Biopharma Services, New Jersey, USA on the Novaseq X Plus 10B 2x150 sequencer. Library preparation was performed using KAPA Hyper Prep kit with PCR (Roche). The DNA was sequenced from both ends, so the output generated were paired-end reads. Total sequencing output for the four *Tribolium* species is presented in Table 6.

PacBio HiFi sequencing and DNA isolation from the flash frozen insect pupae was performed by DNA Sequencing Center/Brigham Young University, Provo, USA. HMW DNA was isolated using the “Genomic-tip 100/G” (Qiagen) kit and the library was prepared by the SMRTbell Express Template Prep Kit 2.0 (Pacific Biosciences). Output of the sequencing is presented in the Table 6.

**Table 6.** Cumulative output of Illumina paired-end reads sequencing and PacBio HiFi sequencing.

	Illumina (151 bp)		PacBioHiFi		
	output (Gb)	sequences	output (Gb)	N50 (Kb)	coverage
<i>T. freemani</i>	10.726	2 x 35 516 583	23.796	14.72	~74.4x
<i>T. madens</i>	5.173	2 x 17 108 408	28.046	15.53	~107.9x
<i>T. confusum</i>	4.972	2 x 16 462 163	27.980	15.68	~111.9x
<i>T. brevicornis</i>	5.464	2 x 18 092 995	25.227	16.40	~64.7x

### 2.2.11. Bioinformatic analyses

#### 2.2.11.1. Defining the satellitomes of species of the genus *Tribolium*

The TAREAN pipeline was used for the detection of tandem repeats (Novák et al. 2017). TAREAN is hosted at the RepeatExplorer platform on the Galaxy server (<https://repeatexplorer-elixir.cerit-sc.cz/galaxy/>). The TAREAN analysis was performed under the following conditions: Cluster size threshold 0.001, perform cluster merging, no filtering of abundant satellite repeats, extra-long queue. Potential novel satDNAs were annotated to corresponding genome assembly (Tfree1.0 for *T. freemani* (Volarić et al., 2022a) or preliminary assemblies (*T. madens*, *T. confusum* and *T. brevicornis*) generated using hifiasm genome assembler (Cheng et al. 2021; Cheng et al. 2022) with the similarity criterion of 70%. Annotations were reviewed using the bioinformatic program Geneious Prime 2023.2.1 (Biomatters Ltd) and duplicated consensus sequences that were present in different TAREAN analyses were excluded from further analyses. The consensus sequences that tandemly repeated 5 or more times were declared to be satDNAs. Novel satDNAs were compared to the sequences present in databases of NCBI GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) using the on-line tool nBLAST (Altschul et al. 1990) and specialized repeat database Repbase (Kohany et al. 2006) (<https://www.girinst.org/replib/>) to determine their potential similarity with already known DNA sequences. All of the downstream analyses (detection of satDNA superfamilies, detection of HOR monomer structures, long-range organization analyses) were performed using the bioinformatic program Geneious Prime 2023.2.1 (Biomatters Ltd). Nucleotide alignments were also done using Geneious Prime, by the Geneious Alignment tool with following conditions: Global alignment with free end gaps, 65% similarity cost matrix, gap open penalty = 12, gap extension penalty = 3.

#### 2.2.11.2. Phylogenetic analyses

To determine whether there are orthologous satDNAs in the genomes of the five *Tribolium* species (*T. castaneum*, *T. freemani*, *T. madens*, *T. confusum* and *T. brevicornis*) a batch search of nucleotide sequences of all *Tribolium* satDNAs was performed using blastn and Discontiguous Megablast algorithms (Morgulis et al. 2008) built into the Geneious Prime program with the default parameters.

To perform the phylogenetic analyses of the orthologous satDNAs, consensus sequences were mapped to the genome assemblies TcasONT for *T. castaneum* satDNAs (Volarić et al. 2024), Tfree1.0 for *T. freemani* satDNAs (Volarić et al. 2022) and corresponding preliminary assemblies for *T. madens*, *T. confusum* and *T. brevicornis* satDNAs with the similarity criterion of 70%, and subsequently extracted. Nucleotide alignments were performed by the Geneious Alignment tool as described above. Phylogenetic trees were generated using the IQ-TREE 2.3.3 algorithm (Minh et al. 2020) accessed through the Galaxy server (<https://usegalaxy.org>) (Abueg et al. 2024). IQ-TREE has an inbuilt model finder that is used to determine the best evolutionary model for generating the maximum

likelihood tree (Kalyanamoorthy et al. 2017). Statistical support for the topology of the phylogenetic trees was determined based on 1000 bootstrap replications using the ultrafast bootstrap (UFBoot) option (Thi Hoang et al. 2017). Phylogenetic trees were visualized and edited using iTOL v6 (Letunic and Bork 2024). Principal component analyses (PCA) were performed using R package FactoMineR (Lê et al. 2008) on distance matrixes generated by R package ape (Paradis and Schliep 2019) and genetic distance model F18 by courtesy of Marin Volarić.

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### 3. RESULTS

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### 3.1. Satellitome of the species *Tribolium freemani*

#### 3.1.1. Detection of satellite DNAs in *T. freemani* genome

To determine the satellitome of the species *T. freemani*, whole genome sequencing was performed using the Illumina platform. The sequencing yielded 71 033 166 paired-end reads with a length of 151 bp (Table 6). The reads were analyzed using the bioinformatics pipeline TAREAN (Novák et al. 2017). The initial TAREAN run was performed with 175 000 randomly selected Illumina paired-end reads, which corresponds to a genomic coverage of 0.1x. However, the reads containing the major, highly abundant satDNA TFREE “clogged” the pipeline, which could only process 44 222 reads, the majority of which matched the satDNA TFREE. For this reason, all subsequent TAREAN analyses were performed with the subset of Illumina paired-end reads from which the major satDNA TFREE was removed, resulting in a pool of 47 255 306 paired-end reads. To maximize the number of satDNAs detected, seven TAREAN analyses were performed with different random subsets of Illumina reads (Table 7). As expected, analyses which were able to process more reads resulted in a larger number of clusters containing possible low-copy satDNAs.

**Table 7.** Summary of the seven different TAREAN analyses performed for the flour beetle *Tribolium freemani*. All analyses were performed with the subsets of reads from which the major satDNA TFREE-containing reads were removed (Juan et al. 1993).

TAREAN clustering analysis	TF1	TF2	TF3	TF4	TF5	TF6	TF7
No. of input reads	175,000	450,000	880,000	1,300,000	1,760,000	1,500,000	1,500,000
No. of analyzed reads	175,000	450,000	880,000	1,300,000	1,433,120	1,135,188	1,411,839
Genome coverage	~0.1x	~0.25x	~0.5x	~0.75	~0.8x	~0.65x	~0.8x
Proportion of reads in top clusters	30%	36%	40%	42%	42%	42%	42%
No. of reads in clusters	83,951 (47.97%)	262,022 (58.23%)	602,466 (68.46%)	981,268 (75.48%)	1,107,469 (77%)	816608 (72%)	1,087,125 (77%)
No. of clusters	12,053	39,925	97,615	159,351	178,152	127,028	175,480
No. of singlets	91,049	187,978	277,534	318,732	325,651	318,580	324,714
No. of satellites	12	27	41	96	98	74	107
No. of high putative satellites	3	7	11	30	21	16	26
No. of low putative satellites	9	20	30	66	77	58	81

The tandem repeat organization of potentially novel satDNAs was investigated by annotating the consensus sequence of each satDNA cluster provided by TAREAN on the *T. freemani* genome



assembly Tfree1.0 (Volarić et al. 2022) with a 70% similarity criterium. The annotations were manually reviewed and the sequences that formed arrays of 5 or more consecutive monomers were declared to be satDNAs. Genomic annotations of the TAREAN output clusters revealed that some of them were repeated as an output in various runs so the duplicates were excluded from further analyses.

### 3.1.2. Analysis of the satellitome of *T. freemani*

Using *in silico* analyses, 134 novel satDNAs were defined in the genome of *T. freemani*. Together with the previously known major satDNA TFREE, referred to as TfrSat01 for the purposes of this study, they form the satellitome of *T. freemani* (Table 8). Consensus sequences of defined satDNAs are presented in Supplementary table 1.

**Table 8.** Satellitome of the flour beetle *Tribolium freemani*. The table contains the main characteristics of satellite DNAs: repeat unit (monomer) lengths, the genome proportion, the A+T base composition of the individual satellite DNAs. The satellite DNAs that show higher order of repeat (HOR) organization are (\*), while the satellite DNAs that belong to the same superfamily are color-coded and labelled with superscripts A, B, C and D.

satDNA	length (bp)	% genome	AT %	satDNA	length (bp)	% genome	AT %	satDNA	length (bp)	% genome	AT %
TfrSat01 <sup>1</sup>	166	31	69.9	TfrSat46	209	0.0019	73.7	TfrSat91	148	0.0010	71.6
TfrSat02	1106	1.5976	67.3	TfrSat47	490	0.0018	77.1	TfrSat92	260	0.0010	71.5
TfrSat03 <sup>A*</sup>	340	5.6957	68.8	TfrSat48	398	0.0017	70.6	TfrSat93	378	0.0010	79.4
TfrSat04 <sup>A</sup>	112	0.0667	68.7	TfrSat49	341	0.0017	71.8	TfrSat94	223	0.0010	74.4
TfrSat05	173	0.0257	71.1	TfrSat50	265	0.0017	74	TfrSat95	230	0.0010	74.8
TfrSat06	143	0.0472	67.1	TfrSat51	206	0.0017	80.6	TfrSat96	173	0.0010	74.6
TfrSat07	166	0.0285	63.3	TfrSat52	355	0.0016	76.3	TfrSat97	184	0.0010	68.5
TfrSat08	120	0.0104	59.2	TfrSat53	369	0.0016	69.4	TfrSat98	281	0.0010	74
TfrSat09	122	0.0125	68.9	TfrSat54	215	0.0016	77.2	TfrSat99	329	0.0010	74.5
TfrSat10	145	0.0188	63.4	TfrSat55	257	0.0016	68.1	TfrSat100	255	0.0010	62.7
TfrSat11	266	0.0257	66.5	TfrSat56	260	0.0016	75.8	TfrSat101	151	0.0153	71.5
TfrSat12	63	0.0188	46	TfrSat57	269	0.0016	75.8	TfrSat102	326	0.0021	71.5
TfrSat13	63	0.0104	47.6	TfrSat58	398	0.0016	77.4	TfrSat103	563	0.0017	73.7
TfrSat14	204	0.0076	62.3	TfrSat59	167	0.0016	73.1	TfrSat104	342	0.0031	67.8
TfrSat15	164	0.0068	68.3	TfrSat60	316	0.0015	71.5	TfrSat105	419	0.0013	72.8
TfrSat16	178	0.0010	70.8	TfrSat61 <sup>D</sup>	155	0.0015	74.8	TfrSat106	362	0.0011	76.2
TfrSat17	63	0.0044	57.1	TfrSat62 <sup>D</sup>	315	0.0013	72.7	TfrSat107	266	0.0010	71.8
TfrSat18	78	0.1459	53.8	TfrSat63	248	0.0015	73	TfrSat108	283	0.0010	63.4

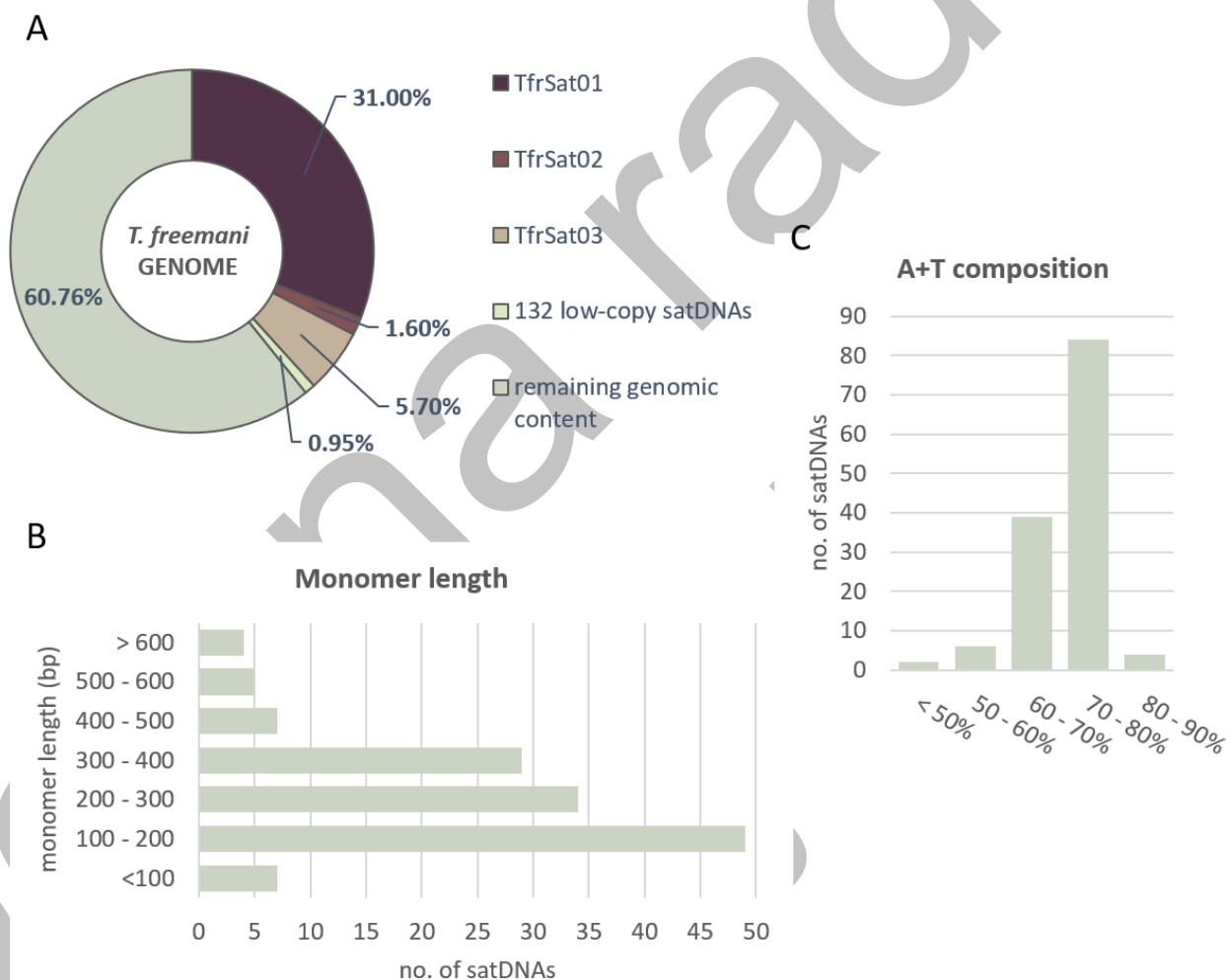
TfrSat19	63	0.0038	58.7	TfrSat64	294	0.0015	78.6	TfrSat109	359	0.0011	76.6
TfrSat20	226	0.2570	80.1	TfrSat65	191	0.0015	62.3	TfrSat110	229	0.0010	76.4
TfrSat21	1006	0.0549	58.4	TfrSat66	390	0.0014	72.3	TfrSat111	115	0.0019	69.6
TfrSat22	333	0.0014	74.2	TfrSat67	99	0.0014	65.7	TfrSat112	321	0.0015	73.5
TfrSat23	167	0.0090	70.1	TfrSat68	193	0.0014	68.9	TfrSat113	279	0.0013	71.3
TfrSat24	164	0.0051	61.6	TfrSat69	149	0.0014	75.2	TfrSat114	176	0.0012	75.6
TfrSat25	154	0.0042	65.6	TfrSat70	174	0.0014	68.4	TfrSat115	156	0.0012	71.2
TfrSat26	416	0.0033	78.1	TfrSat71	173	0.0013	72.3	TfrSat116	159	0.0011	70.4
TfrSat27	180	0.0033	63.9	TfrSat72	196	0.0013	72.4	TfrSat117	301	0.0010	76.1
TfrSat28	362	0.0030	71	TfrSat73	470	0.0013	76.6	TfrSat118	302	0.0010	78.5
TfrSat29	252	0.0029	71.4	TfrSat74	224	0.0013	74.1	TfrSat119	323	0.0010	73.4
TfrSat30	182	0.0028	71.4	TfrSat75	312	0.0013	74	TfrSat120	148	0.0011	64.2
TfrSat31	199	0.0028	70.9	TfrSat76*	180	0.0013	56.1	TfrSat121	426	0.0029	63.1
TfrSat32 <sup>B</sup>	267	0.0027	77.2	TfrSat77*	313	0.0013	76.4	TfrSat122	309	0.0011	73.1
TfrSat33 <sup>B</sup>	504	0.0022	72.8	TfrSat78	283	0.0012	64.7	TfrSat123	423	0.0019	65.7
TfrSat34 <sup>B</sup>	392	0.0013	75.5	TfrSat79	402	0.0012	73.6	TfrSat124	225	0.0017	68.4
TfrSat35	116	0.0027	85.3	TfrSat80	255	0.0012	73.7	TfrSat125	161	0.0015	68.9
TfrSat36	710	0.0024	69.4	TfrSat81	142	0.0011	64.8	TfrSat126	191	0.0015	63.9
TfrSat37	174	0.0024	79.9	TfrSat82	289	0.0011	74	TfrSat127	365	0.0013	70.7
TfrSat38	508	0.0023	73.8	TfrSat83	172	0.0011	65.1	TfrSat128	146	0.0013	73.3
TfrSat39 <sup>C*</sup>	355	0.0021	74.6	TfrSat84	166	0.0011	77.1	TfrSat129	160	0.0013	69.4
TfrSat40 <sup>C</sup>	185	0.0013	75.7	TfrSat85	297	0.0011	74.7	TfrSat130	149	0.0013	71.8
TfrSat41 <sup>C*</sup>	347	0.0015	72.6	TfrSat86	316	0.0011	80.7	TfrSat131	154	0.0013	75.3
TfrSat42 <sup>C</sup>	174	0.0011	73	TfrSat87	262	0.0011	72.1	TfrSat132	262	0.0013	67.6
TfrSat43	97	0.0020	78.4	TfrSat88	569	0.0011	72.8	TfrSat133	288	0.0013	74
TfrSat44	744	0.0020	69.1	TfrSat89	178	0.0011	73	TfrSat134	288	0.0013	76.7
TfrSat45	388	0.0019	75.8	TfrSat90	284	0.0010	76.4	TfrSat135	190	0.0013	64.7

<sup>1</sup>This satDNA corresponds to the major *T. freemani* satDNA, previously characterized Juan et al., 1993.

The 134 novel satDNAs characterized in this study accounted for 8.3% of the total genomic content of *T. freemani*. Most of this is attributed to the satDNAs TfrSat02 and TfrSat03, which account for 1.6% and 5.7% of the genome, respectively. The remaining satDNAs together make up less than 1% of the beetle's genome, so they can be considered low-copy number satDNAs (Figure 8A). Overall, satDNAs dominate the genome of *T. freemani* with a total proportion of 39.2%.

The monomer length of the satDNAs studied in this work varied widely, ranging from 63 bp to 1106 bp (Table 8). Nevertheless, the preferred monomer length is in the range between 100 and 200 pb, with the median of this range being 166 bp. Overall, the monomer length of the majority of the new satDNAs is between 100 and 400 bp (Figure 8B) with a median of 252 bp. In terms of nucleotide composition, the satDNAs of *T. freemani* are A+T rich, with only two satDNAs having an A+T content

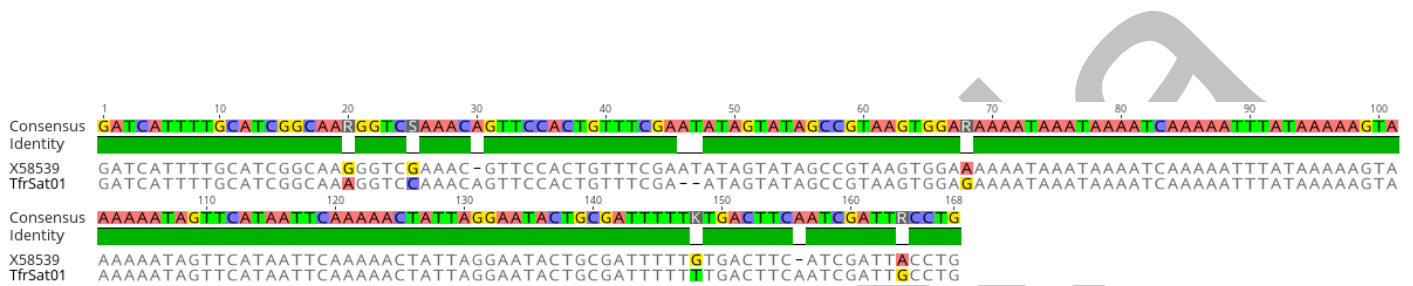
of less than 50%. They strongly favor an A+T composition of more than 60% (127 satDNAs) (Figure 8C) which is in accordance with overall A+T composition of the *T. freemani* genome, which was determined to be 68% (Volarić et al. 2022). The satDNAs were checked for similarities with known transposable elements in RepBase (Kohany et al. 2006) and 69 of them showed partial similarity to various TE, majority of which were DNA transposons (Suppl. table 2).



**Figure 8.** Share of satellitome in the total genomic content (A), monomer length distribution (B) and A+T composition (C) of consensus sequences of the 135 *Tribolium freemani* satellite DNAs.

The major satDNA of *T. freemani* was characterized 30 years ago, and its consensus was derived from five cloned monomers and was deposited to GenBank under the accession number X58539 (Juan et al. 1993). In this research consensus was revised using the genome assembly Tfree1.0. The new consensus TfrSat01 was defined by aligning 800 extracted monomers from the Tfree1.0

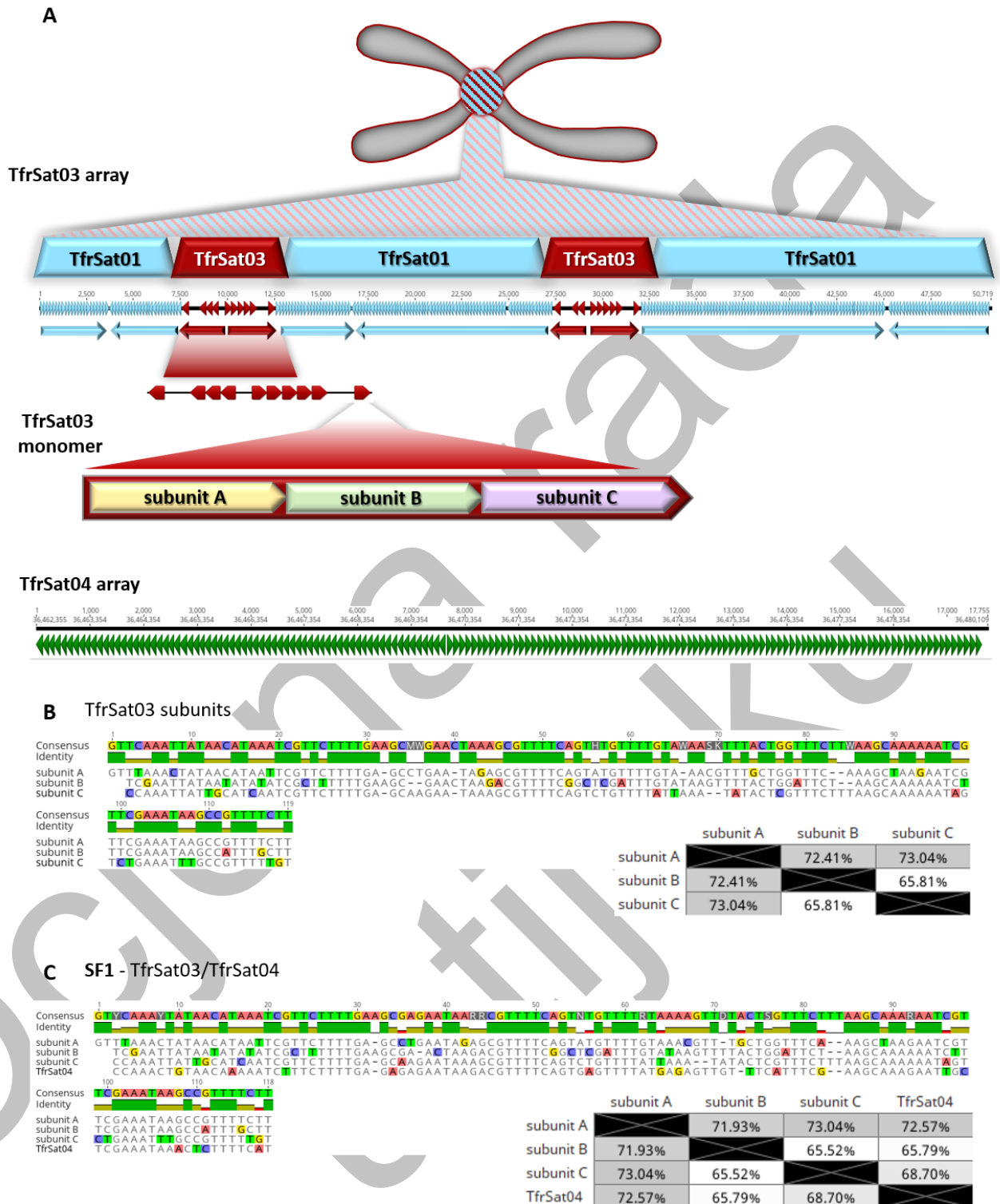
assembly and then compared to the X58539 sequence (Figure 9). It was determined that the two consensus sequences have minimal differences, sharing 94.6% of identical sites.



**Figure 9.** Muscle alignment of the old (X58539) and revised (TfrSat01) consensus sequences of the major satellite DNA of *Tribolium freemani*.

By analyzing the TfrSat01 annotations on the Tfree1.0 genome assembly, it was found that long arrays of major satDNA are organized in the multi-megabase TfrSat01 regions. Within the long arrays, the TfrSat01 monomers are organized into subarrays that differ from each other with respect to monomer orientation (Figure 10A). Such a dyad symmetry of inversely oriented subarrays indicates the potential for the formation of secondary structures such as hairpin and cruciform conformations. Also, TfrSat01 arrays are frequently interrupted by the arrays of TfrSat03 satDNA. Interestingly, the TfrSat03 monomer is a HOR consisting of three subunits similar in nucleotide sequence, showing an average pairwise identity of 70.4% (Figure 10B). In addition, TfrSat03 arrays also exhibit dyad symmetry in their long-range organization, comprising several monomers of TfrSat03 in the forward direction, opposed by several monomers in the reverse direction (Figure 10A).

Further analysis revealed that the TfrSat03 subunits share nucleotide similarity, spanning from 65.8% to 72.6%, with a consensus monomer of TfrSat04 satDNA (Figure 10C), with the length of TfrSat03 subunits corresponding with the length of the TfrSat04 monomer. Due to nucleotide sequence similarity, TfrSat03 and TfrSat04 have been grouped together into the superfamily A of the *T. freemani* satellitome (Table 8). Nevertheless, while the subunits of TfrSat03 form HORs and exhibit a complex organization, the TfrSat04 monomers are neatly tandemly organized in long arrays (Figure 10A).



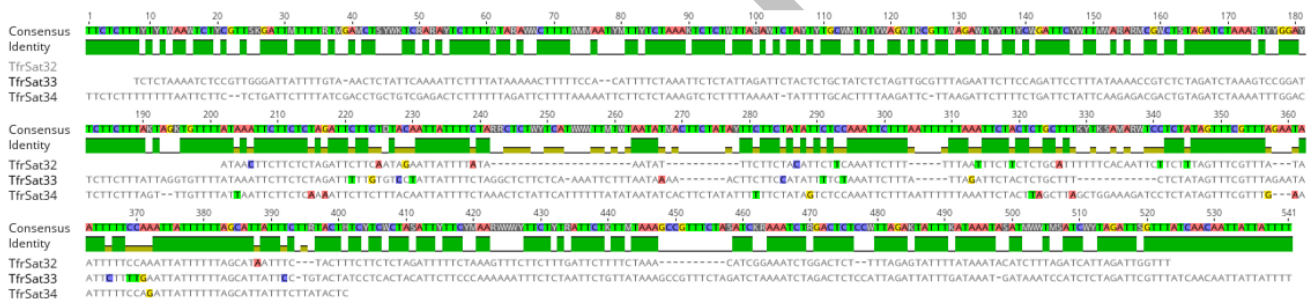
**Figure 10.** A) Schematic representation of *Tribolium freemani* centromeric region containing satellite DNAs TfrSat01 and TfrSat03. The arrays of TfrSat03 are embedded into the TfrSat01 arrays showing dyad symmetry. The arrows show the orientation of the monomers within the TfrSat01 and TfrSat03 arrays. The higher-order repeat structure of TfrSat03 monomer containing the three subunits (A, B, C) is also shown. The organization of TfrSat04 long continuous array in comparison to the embedded TfrSat03 is presented. B) Geneious alignment and distance matrix of the TfrSat03 subunits. C) Geneious alignment and distance matrix of the superfamily SF1 satellite DNAs TfrSat03 and TfrSat04. For TfrSat03, the three subunits (A, B, C) corresponding to the length of the TfrSat04 monomer sequence were compared.



Regarding satDNA mutual similarities, the three additional superfamilies have been characterized, named superfamily B – D. Superfamily B consists of the satDNAs TfrSat32, TfrSat33 and TfrSat34, which are partially similar in their monomeric sequences (Figure 11A). Their monomers share a pairwise identity spanning from 62.8% to 64.7%. Within superfamily C, comprising satDNAs TfrSat39/40/41/42, the relationships between the satDNAs are more complex. All satDNAs share similarities in nucleotide sequences, but TfrSat39 is structured as a dimeric HOR form of a monomeric satDNA TfrSat40 with the subunits having the pairwise identity of 63%. TfrSat41 is also a dimeric HOR with subunits with similar nucleotide composition and pairwise identity of 63.9% (Figure 11B, Figure 12A and 12B). The satDNAs TfrSat61 and TfrSat62 of superfamily D have partial similarity in their consensus monomer sharing 68.4% pairwise identity (Figure 11C).

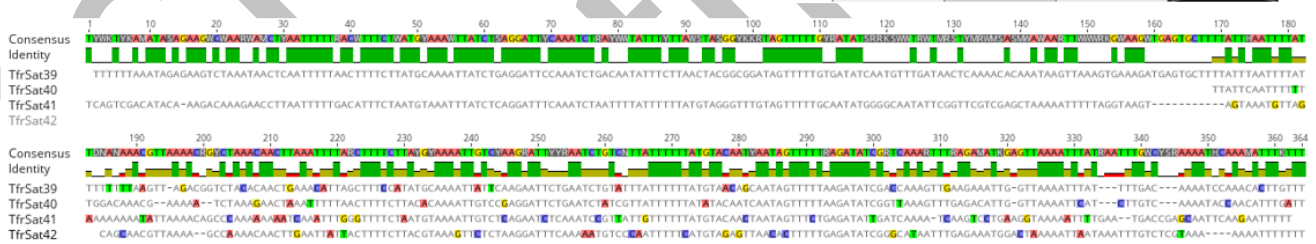
### A Superfamily B - TfrSat32/TfrSat33/TfrSat34

	TfrSat32	TfrSat33	TfrSat34
TfrSat32		62.78%	63.45%
TfrSat33	62.78%		64.72%
TfrSat34	63.45%	64.72%	

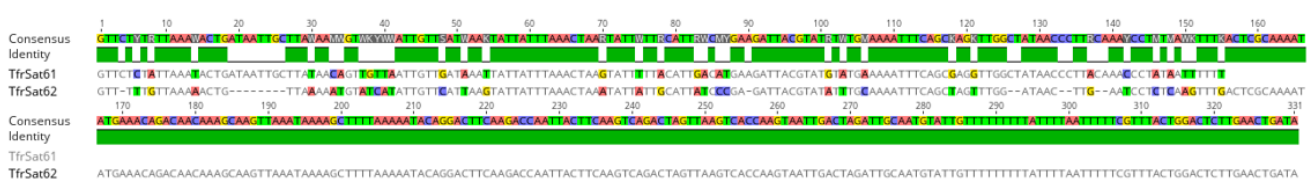


### B Superfamily C - TfrSat39/40/41/42

	TfrSat39	TfrSat40	TfrSat41	TfrSat42
TfrSat39		73.02%	57.06%	59.44%
TfrSat40	73.02%		56.70%	64.80%
TfrSat41	57.06%	56.70%		60.67%
TfrSat42	59.44%	64.80%	60.67%	



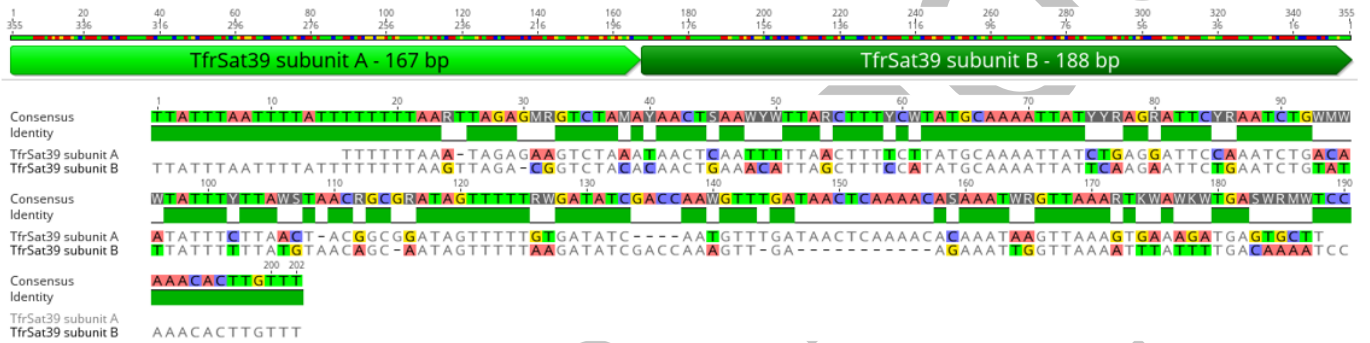
### C Superfamily D – TfrSat61/62, pairwise similarity = 68.4%



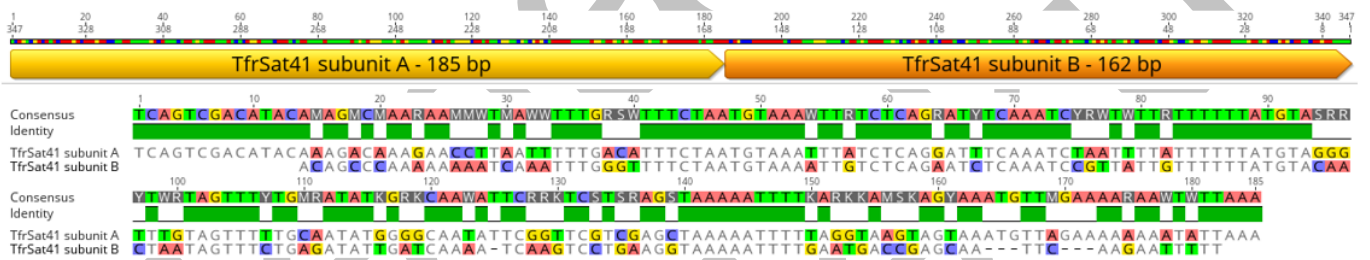
**Figure 11.** Geneious alignments and distance matrices of *Tribolium freemani* satellite DNAs belonging to the superfamily B (A), superfamily C (B) and superfamily D (C).

Regarding the HOR organization of the satDNAs of *T. fremani*, in addition to TfrSat03, TfrSat39 and TfrSat41, there are two additional satDNAs that exhibit HOR structures. The monomer of TfrSat76 consists of two highly similar subunits with a pairwise identity of 82.2% (Figure 12C), while the monomer of TfrSat77 also has two subunits that are 75% similar (Figure 12D).

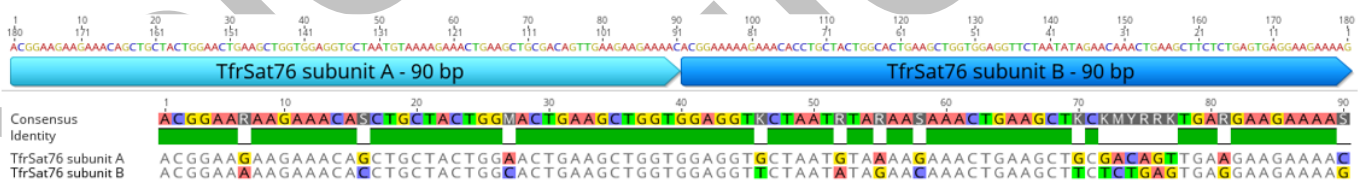
**A TfrSat39 – pairwise identity = 63%**



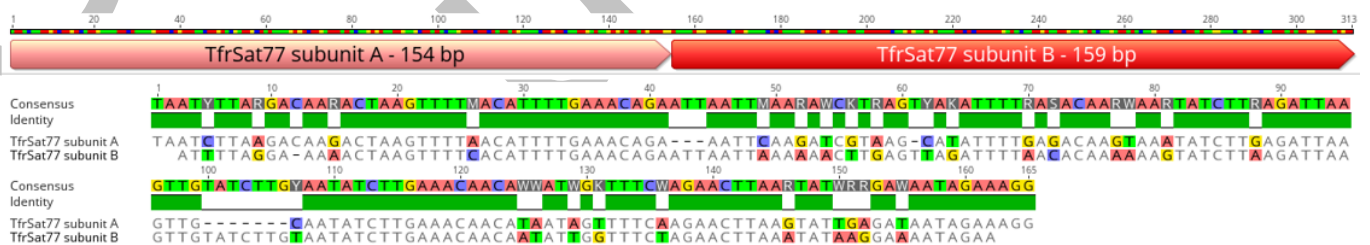
**B TfrSat41 – pairwise identity = 63.9%**



**C TfrSat76 – pairwise identity = 82.2%**



**D TfrSat77 – pairwise identity = 75%**

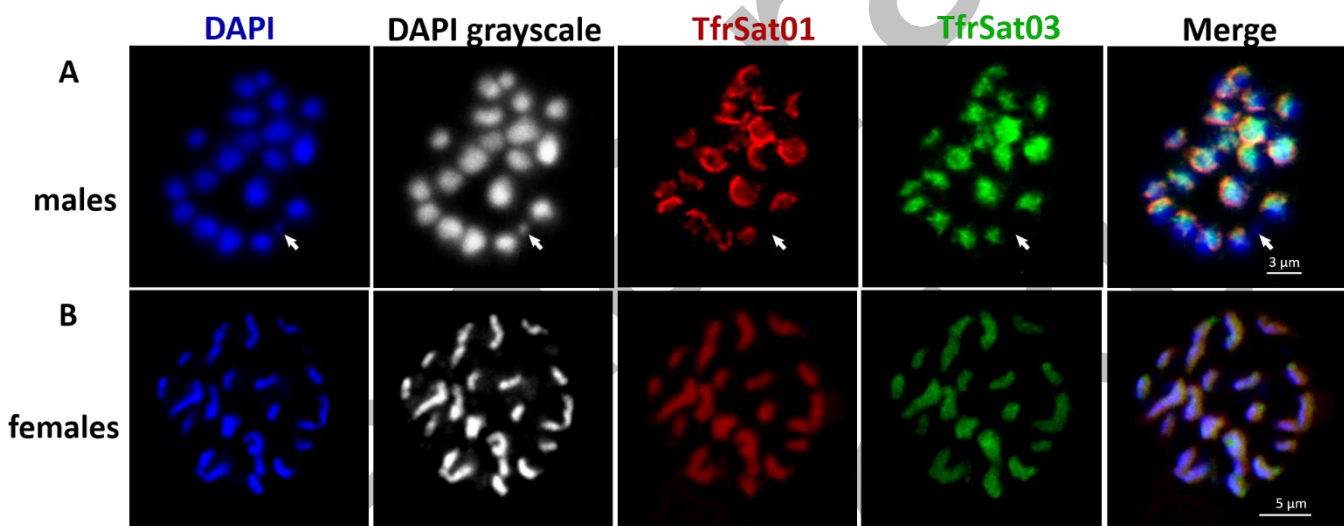


**Figure 12.** Schematic representation of dimeric higher-order repeat structures and Geneious alignments of the subunits of satellite DNAs TfrSat39 (A), TfrSat41 (B), TfrSat76 (C) and TfrSat77 (D) with indicated subunits' pairwise identities.

### 3.1.3. Chromosome localization of the *T. freemani* satellite DNAs

The chromosomal location of the most prominent *T. freemani* satDNAs was determined by FISH experiments.

Since TfrSat01 and TfrSat03 showed a mutually interspersed genomic organization *in silico*, double FISH experiments were performed to confirm their colocalization (Figure 13). On the male chromosome spreads, the TfrSat01 and TfrSat03 signals were detected on 19 chromosomes of the complement in large blocks, mainly in heterochromatic (peri)centromeric regions. Curiously, neither of the two satDNAs showed the signals on the male-specific sex-chromosome  $\gamma_p$  (Figure 13A). However, FISH on female chromosome spreads showed signals on all 20 chromosomes (Figure 13B).

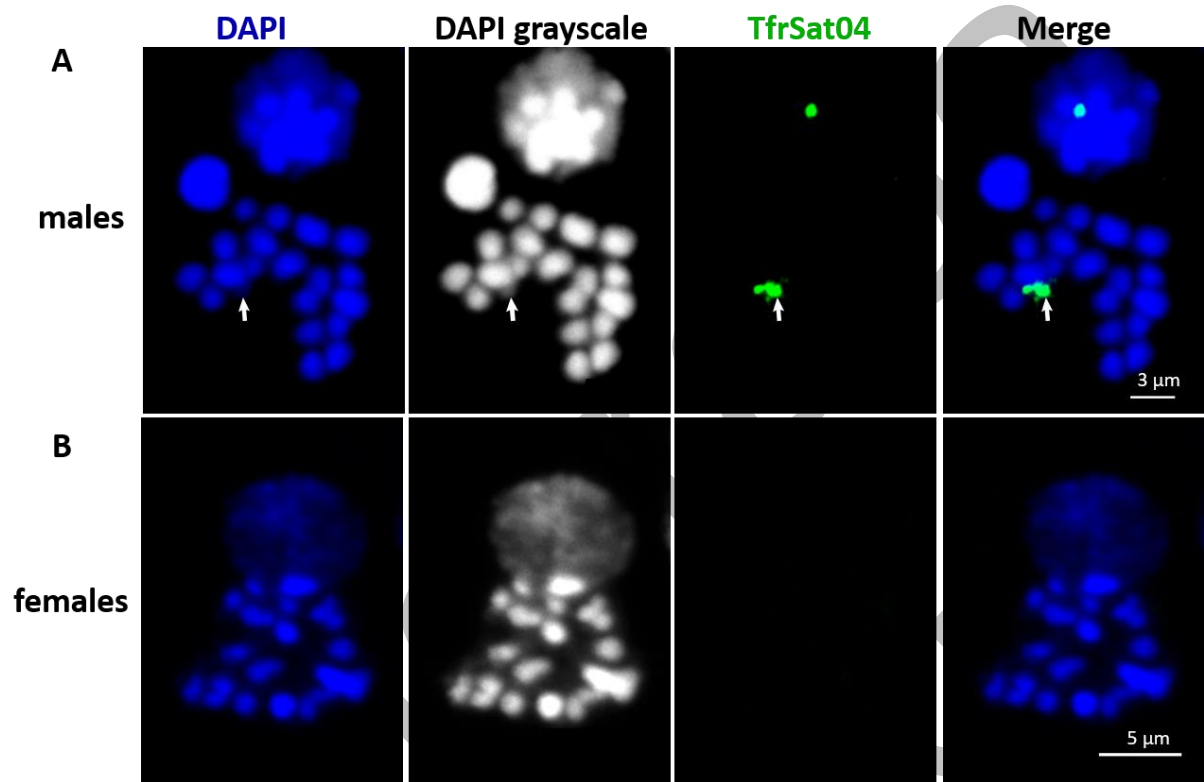


**Figure 13.** Chromosomes of *Tribolium freemani* male (A) and female (B) specimens stained with DAPI and *in situ* hybridized with a Cy3-labelled probe specific for TfrSat01 (red fluorescence) and FITC-labelled probe specific for TfrSat03 (green fluorescence). A DAPI grayscale image is shown to better visualize the contours of the chromosomes. An arrow shows the sex chromosome  $\gamma_p$ . The "merge" panels show the overlap of TfrSat01 and TfrSat03 signals.

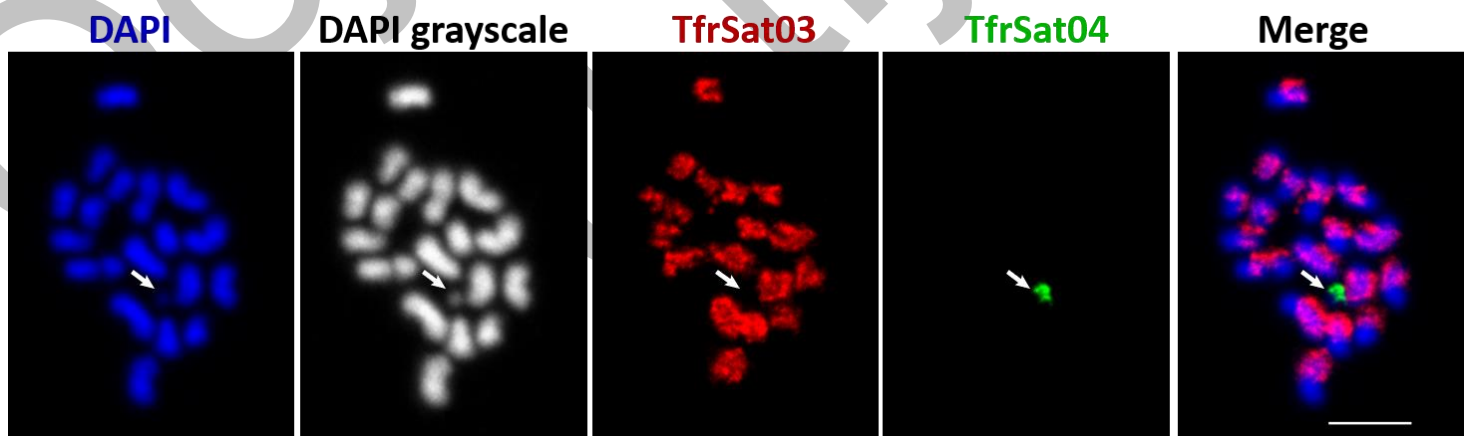
FISH analysis was also performed to identify the position of TfrSat04 satDNA (Figure 14). It revealed that the satDNA TfrSat04 is exclusively present on the  $\gamma_p$  chromosome (Figure 14A). Accordingly, the FISH experiment using the TfrSat04 probe on chromosome spreads isolated from female gonads showed no signals (Figure 14B). Since TfrSat03 and TfrSat04 belong to the same superfamily A and are similar in their nucleotide sequences, the additional double FISH check was performed with TfrSat03 and TfrSat04 specific probes on the male chromosome spreads (Figure 15). In accordance with the previous experiment, no colocalization of the two satDNAs was observed as TfrSat03 was found on all chromosomes except the  $\gamma_p$  chromosome, while TfrSat04 localized on  $\gamma_p$ . These *in situ* experiments revealed that two related satDNAs from the same superfamily differ not



only in their long-range organization but also in their chromosomal localization, with TfrSat04 being specific for the male sex chromosome.

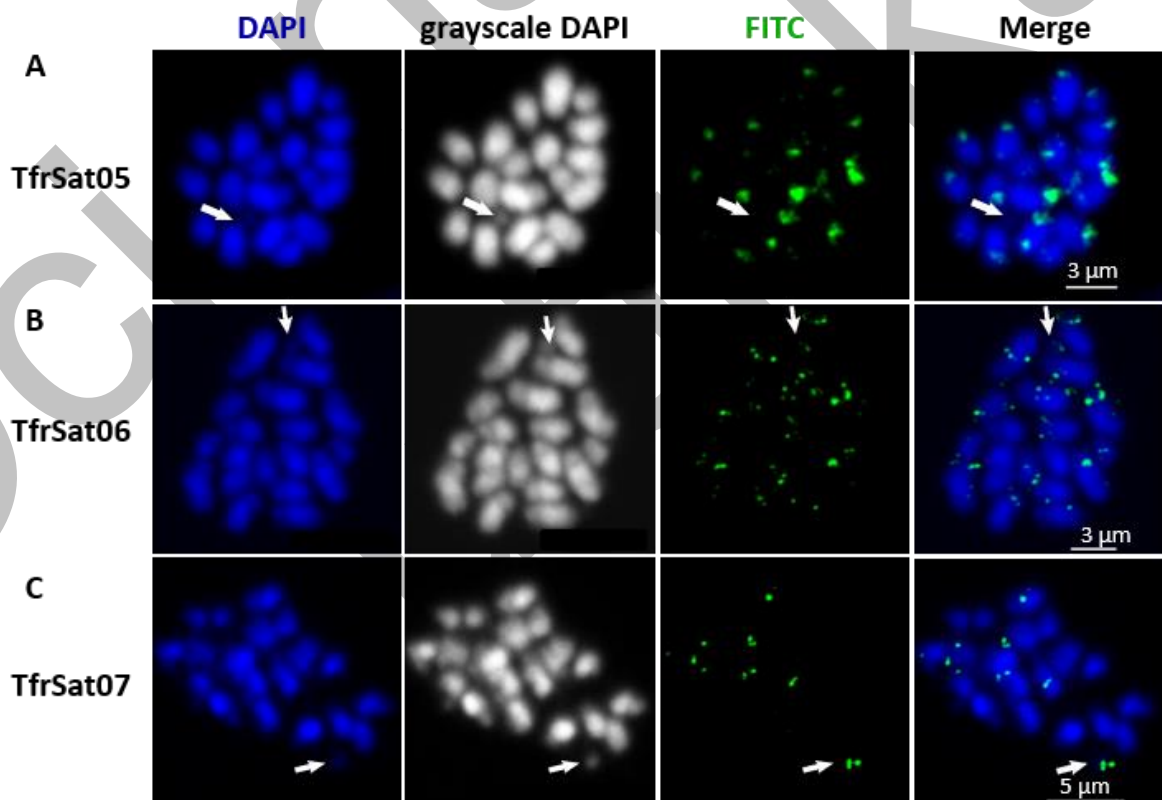


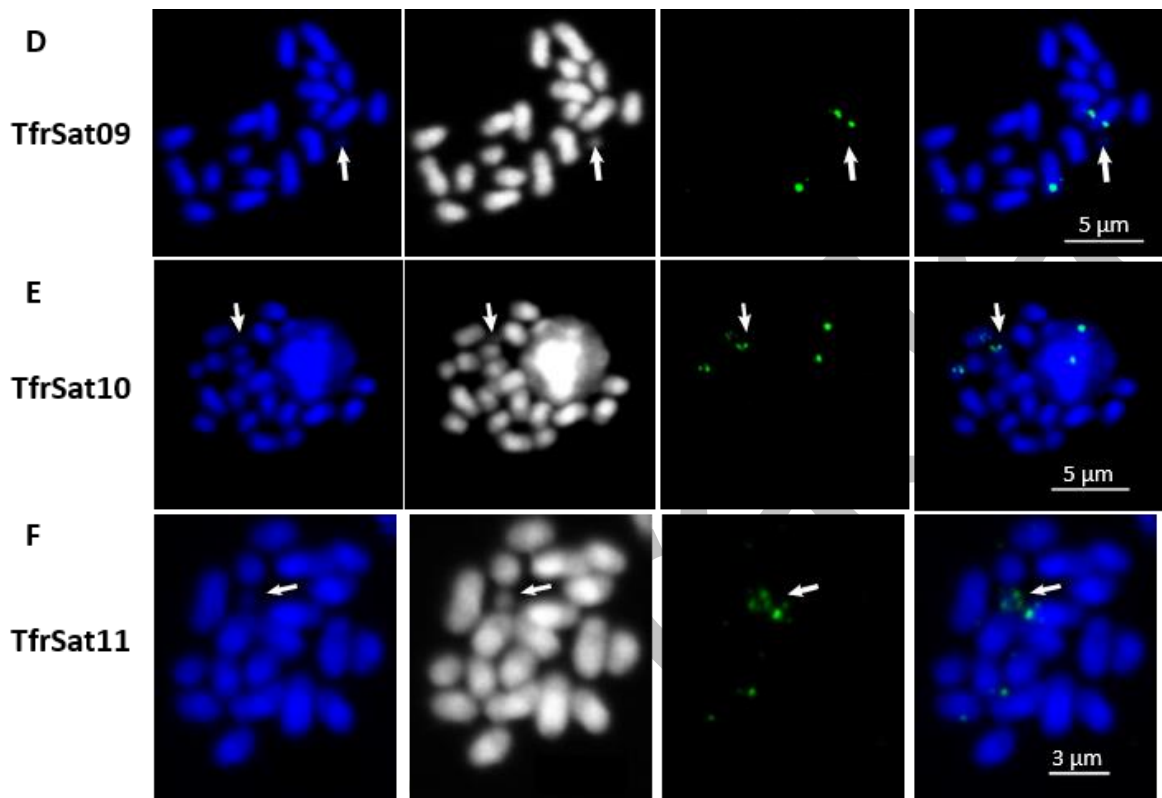
**Figure 14.** Fluorescence *in situ* hybridization of the *Tribolium freemani* male (A) and female (B) chromosomes with TfrSat04 satDNA-specific probe (green FITC signal). The chromosomes counterstained with DAPI (blue) are also shown in grayscale. The white arrow shows the  $y_p$  chromosome.



**Figure 15.** Localization of TfrSat03 and TfrSat04 satDNAs on *T. freemani* male chromosomes (stained in DAPI) obtained by FISH with Cy3-labeled TfrSat03 probe (red fluorescence) and FITC-labelled TfrSat04 probe (green fluorescence). The arrow shows the  $y_p$  chromosome. Scale bar = 5 μm.

FISH experiments were also done for the most conspicuous low-copy-number satDNAs of *T. freemani* (Figure 16). TfrSat05 is located on most chromosomes, but it showed no signal on the  $\gamma_p$  chromosome. In contrast to the highly abundant TfrSat01, which is also found on all chromosomes except on  $\gamma_p$ , the TfrSat05 signals were more dispersed and discrete, and did not extend over the entire heterochromatic regions (Figure 16A). The signals of TfrSat06 were similar to those of TfrSat05, also distributed on the majority of chromosomes (Figure 16B). There is a possibility that there are TfrSat05 and TfrSat06 arrays on additional chromosomes as seen in the annotated genome, but they are too short to be detected by FISH. The satDNA TfrSat07 was located on less than half of the chromosomes of the complement, but interestingly it is present on the  $\gamma_p$  chromosome (Figure 16C). TfrSat09 showed signals on only one pair of chromosomes, which is consistent with the genomic annotation showing only one array of TfrSat09 on chromosome 3 (Figure 16D). The satDNA TfrSat10 was detected on two chromosome pairs and appeared as two distinct signals in the interphase nucleus (Figure 16E). The last low-copy satDNA analyzed by FISH was TfrSat11 with two strong signals on chromosomes and several less intense, scattered signals on two other chromosome pairs (Figure 16F). The FISH results for all of the examined low-copy-number satDNAs are consistent with the genomic annotations in the Tfree1.0 assembly.





**Figure 16.** Chromosomes of *Tribolium freemani* male specimens hybridized *in situ* with a FITC-labelled probe specific for TfrSat05 (A), TfrSat06 (B), TfrSat07 (C), TfrSat09 (D), TfrSat10 (E) and TfrSat11 (F). An arrow shows the sex chromosome  $\gamma_p$ .

### 3.2. Satellitome of the species *Tribolium madens*

#### 3.2.1. Detection of satellite DNAs in *T. madens* genome

The satellitome of the species *T. madens* was determined using whole genome sequence reads obtained from the Illumina platform. The sequencing yielded 33 902 390 paired-end reads with a length of 151 bp (Table 6). Six different TAREAN analyses were performed (Table 9). The first analysis TM1 was performed with a random subsample of 168 290 pair-end reads, corresponding to approximately 0.1x coverage with the two major satDNAs included. Further analyses were performed on the subset of 21 280 718 original Illumina reads missing the two major satDNAs MAD1 and MAD2 to maximize the total amount of satDNAs that could be mined.

**Table 9.** Summary of the results of six different TAREAN analyses performed for the black flour beetle *Tribolium madens*.

TAREAN clustering analysis	TM1	TM2*	TM3*	TM4*	TM5*	TM6*
No. of input reads	168,290	168,402	416,172	840 000	1 260 000	1,684,340
No. of analyzed reads	168,290	168,402	416,172	832,478	1,248,506	1,684,340
Genome coverage	~0.1x	~0.1x	~0.25x	~0.5x	~0.75	~1x
Proportion of reads in top clusters	48%	19%	22%	25%	28%	30%
No. of reads in clusters	98,129 (58.31%)	62,627 (37.19%)	208,307 (50.05%)	539,831 (64.85%)	932,534 (74.69%)	1,378,784 (81.86%)
No. of clusters	7,057	13,176	48,098	124,970	204,038	274,966
No. of singlets	70,161	105,775	207,865	292,647	315,972	305,556
No. of satellites	5	8	16	25	77	119
No. of high putative satellites	3	2	7	8	9	18
No. of low putative satellites	2	6	9	17	68	101

\*analyses performed with the subset of reads missing the major satDNAs MAD1 and MAD2 (Ugarković et al. 1996b)

To determine whether the potential satDNAs are indeed tandemly repeated, the consensus sequences provided by TAREAN were annotated to the preliminary assembly of *T. madens*, which was assembled using PacBio HiFi reads (unpublished results) applying the 70% similarity criterion. All sequences that showed a tandem organization of 5 or more repeating monomers were declared to be satDNAs.

### 3.2.2. Analysis of the satellitome of *T. madens*

In total, 122 new satDNAs were found in the genome of the black flour beetle *T. madens*. For the purpose of sequential numeration of newly discovered satDNAs, the two already characterized major satDNAs MAD1 and MAD2 (Ugarković et al. 1996b) are referred to as TmaSat01 and TmaSat02, respectively. Altogether, the satellitome of *T. madens* consists of 124 satDNAs (Table 10) whose consensus sequences are presented in Supplementary table 1.

**Table 10.** Satellitome of the black flour beetle *Tribolium madens*. The table contains the main characteristics of detected satellite DNAs: repeat unit (monomer) lengths, the genome proportion, the A+T base composition of the individual satellite DNAs. The satellite DNAs with higher-order-repeats (HORs) are shaded in a darker shade of green and marked with an asterisk.

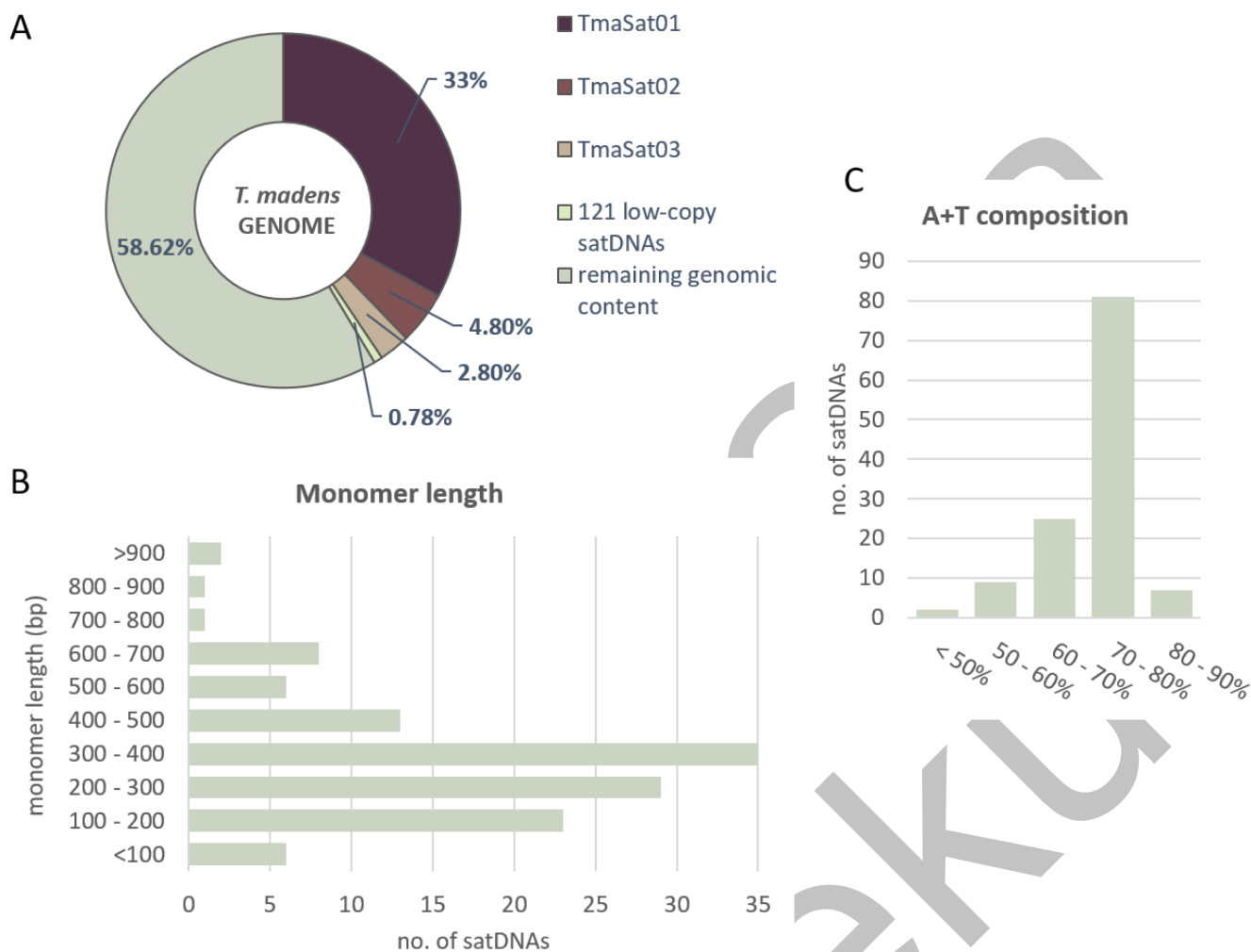
satDNA	length (bp)	% genome	AT%	satDNA	length (bp)	% genome	AT%	name	length (bp)	% genome	AT%
TmaSat01 <sup>1</sup>	225	33	74.2	TmaSat43*	174	0.0017	55.2	TmaSat84	414	0.0015	78.3
TmaSat02 <sup>1</sup>	711	4.8	69.6	TmaSat44	283	0.0017	75.6	TmaSat85	433	0.0015	76.4
TmaSat03	1264	2.8	67.6	TmaSat45	264	0.0016	59.5	TmaSat86	433	0.0015	70
TmaSat04	123	0.0081	63.4	TmaSat46	209	0.0016	77.5	TmaSat87	276	0.0013	67
TmaSat05	145	0.0050	62.8	TmaSat47	343	0.0016	64.1	TmaSat88	580	0.0013	75.3
TmaSat06	166	0.0199	71.1	TmaSat48	312	0.0015	67.6	TmaSat89	453	0.0013	75.3
TmaSat07	306	0.0118	75.8	TmaSat49	280	0.0014	69.3	TmaSat90	291	0.0013	69.4
TmaSat08	363	0.0170	46.6	TmaSat50	214	0.0014	82.2	TmaSat91	342	0.0013	74
TmaSat09	99	0.0057	64.6	TmaSat51	206	0.0014	75.2	TmaSat92	212	0.0012	81.1
TmaSat10	334	0.0059	76	TmaSat52	543	0.0014	78.1	TmaSat93	643	0.0011	81.8
TmaSat11*	102	0.0051	53.9	TmaSat53	297	0.0014	65.7	TmaSat94	173	0.0011	65.9
TmaSat12	132	0.0009	62.1	TmaSat54	272	0.0014	72.8	TmaSat95	212	0.0011	67
TmaSat13	63	0.0150	57.1	TmaSat55	211	0.0014	81	TmaSat96	195	0.0011	65.6
TmaSat14	90	0.0033	53.3	TmaSat56	366	0.0014	71.6	TmaSat97	317	0.0011	71.9
TmaSat15	692	0.5000	69.1	TmaSat57*	389	0.0014	71.7	TmaSat98	303	0.0011	70.6
TmaSat16*	450	0.0180	70.9	TmaSat58	174	0.0013	52.9	TmaSat99	63	0.0011	42.9
TmaSat17	55	0.0036	78.2	TmaSat59	394	0.0012	77.9	TmaSat100	228	0.0010	79.4
TmaSat18	206	0.0012	70.9	TmaSat60	269	0.0012	67.7	TmaSat101	658	0.0010	78.3
TmaSat19	264	0.0011	78.4	TmaSat61	221	0.0012	65.6	TmaSat102	382	0.0010	77.7
TmaSat20	122	0.0019	68	TmaSat62	301	0.0012	79.7	TmaSat103	164	0.0009	71.3
TmaSat21	477	0.0009	76.9	TmaSat63	336	0.0011	77.4	TmaSat104	303	0.0009	73.3
TmaSat22	161	0.0052	71.4	TmaSat64	331	0.0011	75.2	TmaSat105	171	0.0009	67.3
TmaSat23	525	0.0046	76.8	TmaSat65	353	0.0011	70.8	TmaSat106	245	0.0009	72.2
TmaSat24	451	0.0037	76.1	TmaSat66	165	0.0011	73.3	TmaSat107	303	0.0009	58.7
TmaSat25	259	0.0030	75.7	TmaSat67	313	0.0011	72.5	TmaSat108	436	0.0009	74.8
TmaSat26	687	0.0047	75.5	TmaSat68	451	0.0010	74.1	TmaSat109	333	0.0009	73
TmaSat27	133	0.0019	57.1	TmaSat69	279	0.0010	68.5	TmaSat110	323	0.0009	74.9

<b>TmaSat28*</b>	418	0.0018	71.1	<b>TmaSat70</b>	321	0.0010	75.1	<b>TmaSat111</b>	300	0.0009	74.3
<b>TmaSat29</b>	238	0.0017	70.2	<b>TmaSat71</b>	327	0.0010	70	<b>TmaSat112</b>	264	0.0009	54.2
<b>TmaSat30</b>	333	0.0017	76	<b>TmaSat72</b>	1275	0.0051	73.3	<b>TmaSat113</b>	158	0.0009	81.6
<b>TmaSat31</b>	288	0.0017	76.4	<b>TmaSat73</b>	260	0.0025	74.2	<b>TmaSat114</b>	159	0.0009	67.9
<b>TmaSat32</b>	382	0.0015	74.6	<b>TmaSat74</b>	859	0.0025	74.6	<b>TmaSat115</b>	135	0.0008	68.9
<b>TmaSat33</b>	123	0.0032	74	<b>TmaSat75</b>	395	0.0024	81.5	<b>TmaSat116</b>	328	0.0008	78
<b>TmaSat34</b>	216	0.0028	71.8	<b>TmaSat76</b>	615	0.0019	71.4	<b>TmaSat117</b>	163	0.0008	63.2
<b>TmaSat35</b>	141	0.0024	78	<b>TmaSat77</b>	624	0.0019	72.1	<b>TmaSat118</b>	334	0.0007	73.4
<b>TmaSat36*</b>	300	0.0024	77.3	<b>TmaSat78</b>	640	0.0019	74.2	<b>TmaSat119</b>	204	0.0007	81.4
<b>TmaSat37</b>	365	0.0021	77.3	<b>TmaSat79</b>	392	0.0019	71.7	<b>TmaSat120</b>	188	0.0007	72.3
<b>TmaSat38</b>	629	0.0019	78.4	<b>TmaSat80</b>	528	0.0016	74.1	<b>TmaSat121</b>	259	0.0007	74.5
<b>TmaSat39</b>	343	0.0019	75.2	<b>TmaSat81*</b>	351	0.0016	76.4	<b>TmaSat122</b>	133	0.0007	73.7
<b>TmaSat40</b>	342	0.0019	73.1	<b>TmaSat82</b>	547	0.0016	73.9	<b>TmaSat123</b>	454	0.0007	78.2
<b>TmaSat41</b>	408	0.0017	75.2	<b>TmaSat83</b>	562	0.0016	73.1	<b>TmaSat124</b>	79	0.0007	65.8
<b>TmaSat42</b>	413	0.0017	75.5								

<sup>†</sup>The satDNAs detected previously in Ugarković et al. 1996b

The 122 novel satDNAs make up 3.6% of the total genomic content of *T. madens*. The majority of it belongs to the satDNA TmaSat03 which accounts for 2.8% of the genome, while the percentage of the cumulative remaining satDNA does not exceed 1%. The total satellitome percentage of *T. madens* is 41.4%, with TmaSat01 dominating the genome with 33% and TmaSat02 being the second most abundant with 4.8% (Figure 17A). 79 satDNAs of the satellitomes have been found to share similarities with a repeat element from the RebBase data base, the majority of which are DNA transposons (Suppl. table 3).

The satDNAs of *T. madens* exhibit a wide range of monomer lengths, ranging from 55 to 1275 bp, with a median length of 303 bp. As it was the case with satDNAs of *T. freemani*, the majority of the novel satDNAs of *T. madens* have a monomer unit between 100 – 400 bp long, with a preferred length spanning between 300 and 400 bp (Figure 17B). Regarding the base composition of satDNAs, they are A+T rich, with only one satellite with the A+T content of less than 50%. The majority of satDNAs (88 of 124) have the A+T content of more than 70% (Figure 17C).



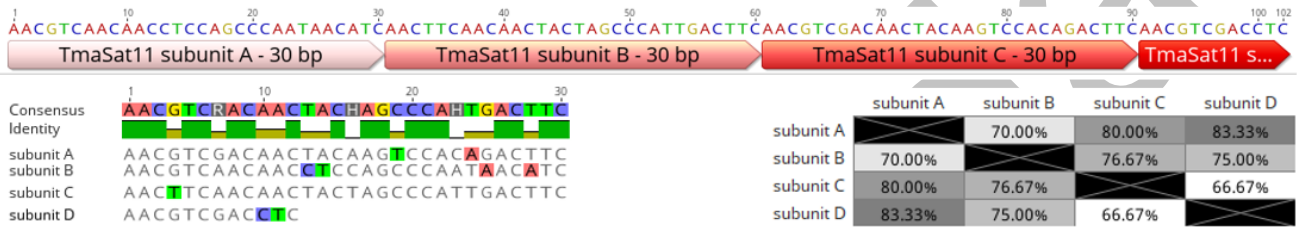
**Figure 17.** Share of satellitome in the total genomic content (A), monomer length distribution (B) and A+T composition (C) of consensus sequences of the 124 *Tribolium madens* satellite DNAs.

Several of the *T. madens* satDNAs show HOR organization of their monomer unit. The monomer of TmaSat11 has a 30 bp pairs sequence repeated three times with a 12 bp tail of the same sequence at the end of the consensus, with pairwise similarity ranging from 66.7% to 83.3% (Figure 18A). A similar situation is observed within the 450 bp long monomer unit of TmaSat16 that consists of a 134 bp sequence that repeats three times, with a similarity of 73.1% to 78.7%. The end of a monomer also shows similarities with a repeated subunit (Figure 18B). TmaSat28 and TmaSat36 satDNAs have highly conserved segments, being 109 bp (Figure 18C) and 103 bp (Figure 18D) long, respectively, that are repeated twice in a monomer. The two satDNAs do not have typical HOR organization, but could be considered complex monomer units because of their conserved segments. Dimeric HOR organization is recognized in the structure of TmaSat43 (Figure 18E) and TmaSat81 (Figure 18G). The similarity between subunits of TmaSat43 is 65.5% but their core is highly conserved, showing a similarity of 93.9% (Figure 18E). Subunits of TmaSat81 share a similarity of 82% (Figure 18G).

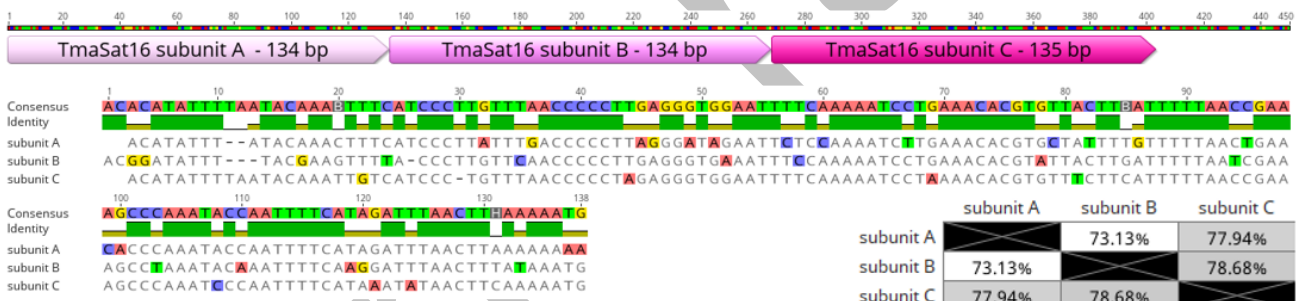


The TmaSat57 monomer shows the most complex structure, being a hexameric HOR based on ~66 bp long subunits (Figure 18F).

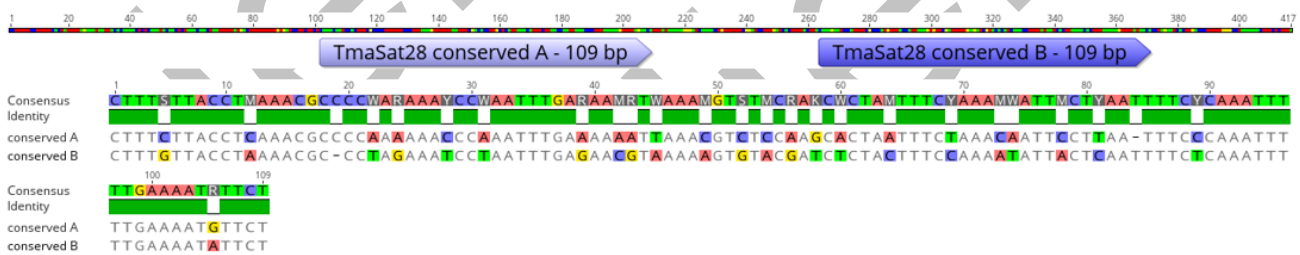
### A TmaSat11 – 102 bp



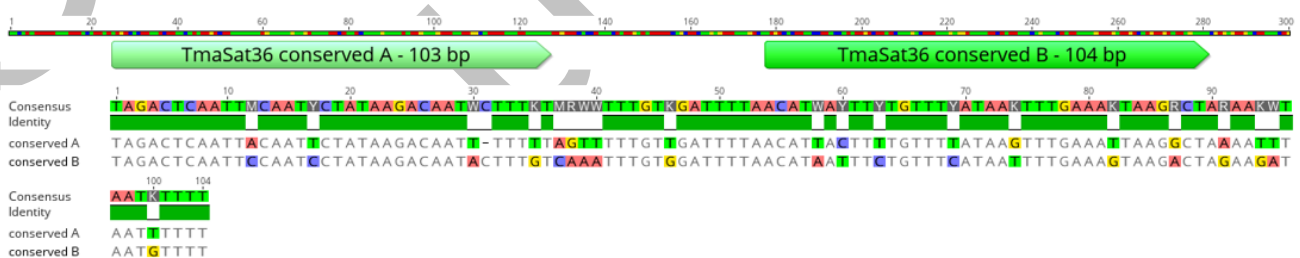
### B TmaSat16 – 450 bp



### C TmaSat28 – pairwise similarity of conserved regions = 76.1%



### D TmaSat36 – pairwise similarity of conserved regions = 79.8%

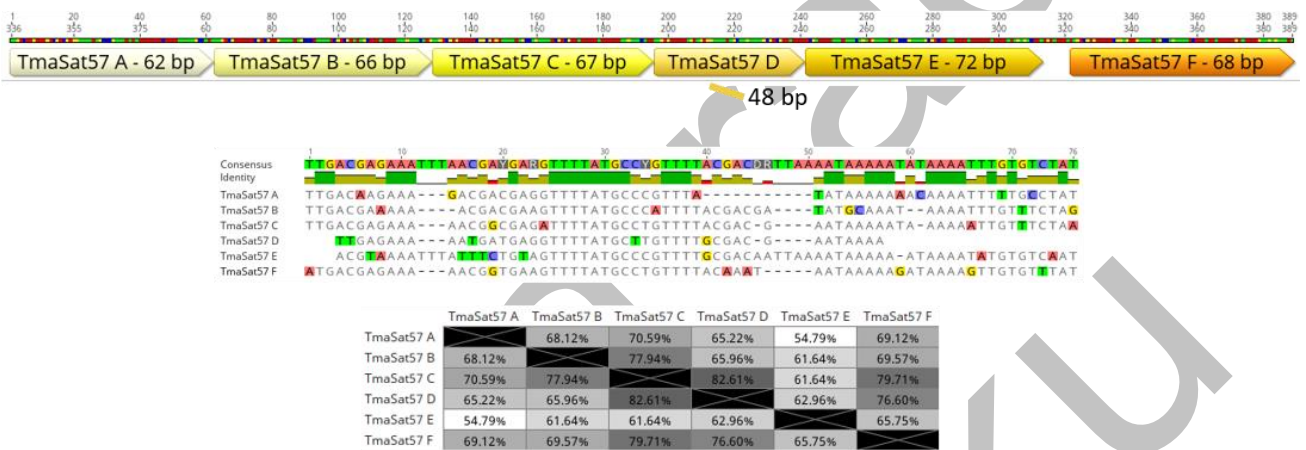




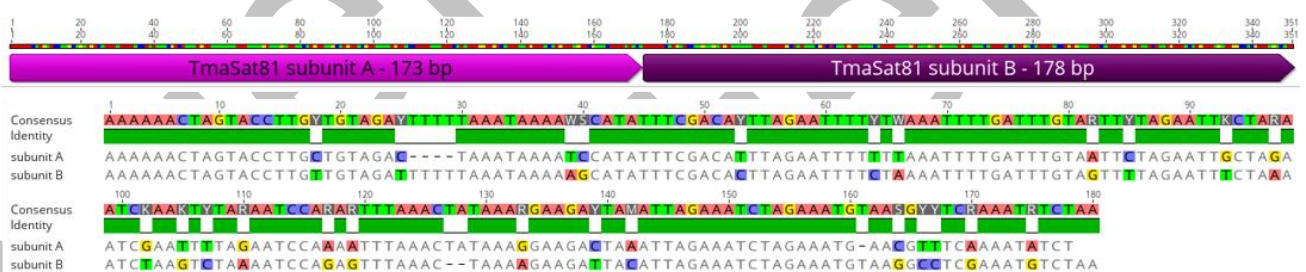
**E TmaSat43 – pairwise similarity subunits = 65.5%; pairwise similarity conserved regions = 93.9%**



**F TmaSat57 – 398 bp**



**G TmaSat81 – pairwise similarity = 82%**

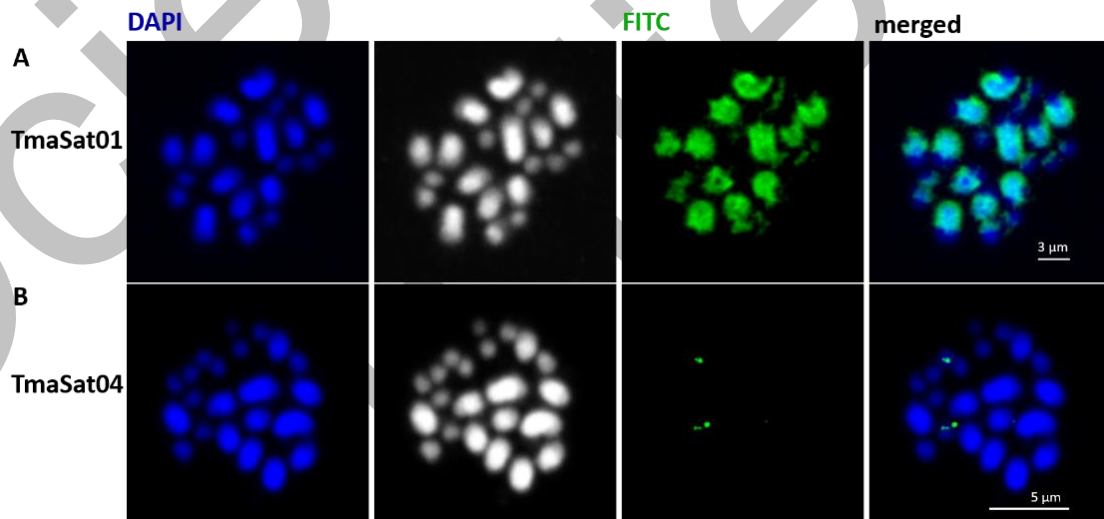


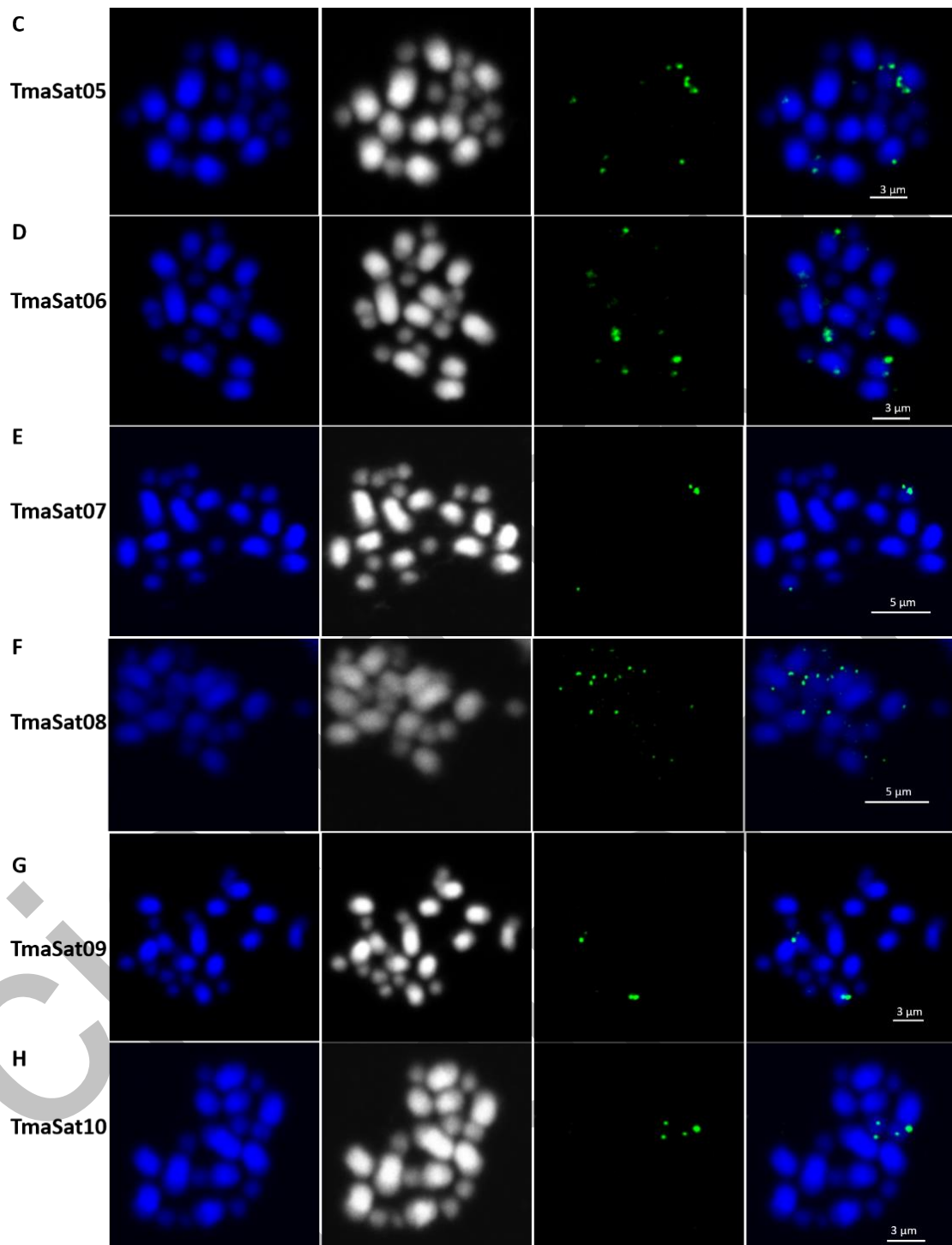
**Figure 18.** Higher-order repeat structures of satellite DNAs TmaSat11 (A), TmaSat16 (B), TmaSat28 (C), TmaSat36 (D), TmaSat43 (E), TmaSat57 (F) and TmaSat81 (G). For each satellite DNA there is a schematic representation of higher-order repeat structure and the alignment of the subunits with pairwise identities indicated.

### 3.2.3. Chromosome localization of the *T. madens* satellite DNAs

FISH experiments were performed to localize seven newly discovered satDNAs on *T. madens* chromosomes. Hybridization with a probe specific for the most abundant *T. madens* satellite, TmaSat01, was included in the FISH analysis as a positive control. It must be emphasized that the karyotype of *T. madens* consists of  $2n=20$  chromosomes, which differ considerably in size. The karyotype also includes a variable number of small supernumerary chromosomes (usually between 1-3), that are very similar in size and appearance to the small chromosomes of the regular complement, and it is practically impossible to distinguish them.

TmaSat01 is present on (peri)centromeric regions on all of the chromosomes ( $2n=20$  + supernumeraries) (Figure 19A). As it is expected, novel satDNAs are present on fewer chromosomes as they are low-copy satDNAs in regards to their abundance (Table 10). The satDNAs TmaSat04 (Figure 19B), TmaSat07 (Figure 19E), TmaSat09 (Figure 19G) and TmaSat10 (Figure 19H) are all located only on one pair of chromosomes in the complement. Contrary to the aforementioned satDNAs, the satDNA TmaSat05 (Figure 19C), TmaSat06 (Figure 19D) and TmaSat08 (Figure 19F) are present on a larger number of chromosomes, approximately on half of the chromosome complement. These cytogenetic results are consistent with the *in silico* analysis that shows all of the monomers of TmaSat04, TmaSat07, TmaSat09 and TmaSat10 tandemly organized in a single genomic array, while the remaining satDNAs are dispersed throughout more contigs.





**Figure 19.** Chromosomes of *Tribolium madens* male specimens counterstained by DAPI and hybridized with a FITC-labelled probe (green signals) specific for TmSat01 (A), TmSat04 (B), TmSat05 (C), TmSat06 (D), TmSat7 (E), TmSat08 (F), TmSat09 (G) and TmSat10 (H) satellite DNAs. DAPI-counterstained chromosomes (blue) are also shown in grayscale to better visualize the chromosome contours. The last panels show merged images of DAPI-stained chromosomes and FITC-labelled satellite DNA signals (green).

### 3.3. Satellitome of the species *Tribolium confusum*

#### 3.3.1. Detection of satellite DNAs in *T. confusum* genome

Using the 151 bp short reads generated by whole genome sequencing with the Illumina platform, the satellitome of *T. confusum* was characterized. The total number of paired-end reads sequenced was 32 571 752 (Table 6). TAREAN analyses TC1 and TC2 were performed with the random subset of total Illumina reads, but the major satDNA (Plohl et al. 1993) was shown to be too abundant for the pipeline. Because of that, further analyses (TC3 – TC7) were performed with the subsample of reads from which the major satDNA was filtered (28 311 462 reads in total) (Table 11).

**Table 11.** Summary of the results of seven different TAREAN analyses performed for the confused flour beetle *Tribolium confusum*.

TAREAN clustering analysis	TC1*	TC2*	TC3*	TC4*	TC5*	TC6*	TC7*
No. of input reads	160,346	400,808	160,420	401,440	802,724	1,204,028	1,605,468
No. of analyzed reads	160,346	325,504	160,420	401,440	802,724	1,204,028	1,605,468
Genome coverage	0.1x	~0.2x	0.1x	~0.25x	~0.5x	~0.75	~1x
Proportion of reads in top clusters	26%	31%	17%	24%	28%	31%	33%
No. of reads in clusters	70,100 (43.72%)	160,446 (40.03%)	59,044 (36.80%)	184,331 (45.92%)	445,316 (55.48%)	753,774 (62.60%)	1,095,528 (68.24%)
No. of clusters	10,458	23,048	12,099	34,861	85,192	144,922	208,881
No. of singlets	90,246	165,058	101,376	217,109	357,408	450,254	509,940
No. of satellites	10	18	12	21	40	82	94
No. of high putative satellites	6	11	6	11	19	33	29
No. of low putative satellites	4	7	6	10	21	49	65

\*analyses performed with the subset of reads from which the major satDNA (Plohl et al. 1993) was removed

The consensus sequences of potential novel satDNAs were annotated to the preliminary draft genome assembly, assembled using PacBio HiFi reads (unpublished results), with the similarity criterion of 70%. All of the sequences that were tandemized in arrays of 5 or more monomers were defined as satDNAs.

### 3.3.2. Analysis of the satellitome of *T. confusum*

Using previously described *in silico* methods, 107 new satDNAs were characterized in the genome of *T. confusum*. Counting the previously defined major satDNA (Plohl et al. 1993) (referred to as TcoSat01 in this work), the satellitome of *T. confusum* consists of 108 different satDNAs (Table 12). The consensus sequences of satDNA monomer are shown in Supplementary table 1.

**Table 12.** Satellitome of the confused flour beetle *Tribolium confusum*. The table contains the main characteristics of satellite DNAs: repeat unit (monomer) lengths, the genome proportion, the A+T base composition of the individual satellite DNAs. The satellite DNAs with higher-order-repeats (HORs) are marked with an asterisk (\*), while the satDNAs that belong to the same superfamily are color-coded and labelled with superscripts A, B, C, D, E, F and G.

satDNA	length (bp)	% genome	AT%	satDNA	length (bp)	% genome	AT%	satDNA	length (bp)	% genome	AT%
TcoSat01 <sup>1</sup>	158	13	73.4	TcoSat37 <sup>C</sup>	64	0.0037	65.6	TcoSat73	181	0.0019	74
TcoSat02 <sup>A</sup>	183	2.2500	73.2	TcoSat38	398	0.0035	65.1	TcoSat74	62	0.0019	77.4
TcoSat03 <sup>A</sup>	183	0.4450	74.9	TcoSat39	211	0.0028	69.7	TcoSat75	437	0.0019	68.4
TcoSat04	185	1.2000	73	TcoSat40	170	0.0026	72.4	TcoSat76	242	0.0018	73.6
TcoSat05	127	0.1800	78	TcoSat41	153	0.0026	72.5	TcoSat77	237	0.0017	75.1
TcoSat06	139	0.0220	60.4	TcoSat42	268	0.0023	70.9	TcoSat78	154	0.0017	76
TcoSat07*	120	0.0135	55	TcoSat43	491	0.0022	72.5	TcoSat79	174	0.0017	73
TcoSat08	547	0.0150	73.1	TcoSat44	176	0.0022	73.3	TcoSat80 <sup>G</sup>	163	0.0017	82.2
TcoSat09	120	0.0080	60.8	TcoSat45	177	0.0022	56.5	TcoSat81 <sup>G</sup>	432	0.0021	79.9
TcoSat10	177	0.2600	72.9	TcoSat46	379	0.0016	63.1	TcoSat82 <sup>G</sup>	151	0.0014	72.2
TcoSat11	655	0.0296	63.1	TcoSat47	184	0.0016	76.6	TcoSat83 <sup>G</sup>	212	0.0014	70.8
TcoSat12	600	0.0200	69.8	TcoSat48	181	0.0015	76.2	TcoSat84 <sup>G</sup>	134	0.0011	79.9
TcoSat13	182	0.0061	72.5	TcoSat49 <sup>D</sup>	123	0.0015	74.8	TcoSat85 <sup>G</sup>	302	0.0044	78.5
TcoSat14	90	0.0104	66.7	TcoSat50 <sup>D</sup>	562	0.0037	73.7	TcoSat86	150	0.0017	73.3
TcoSat15 <sup>B</sup>	141	0.0131	76.6	TcoSat51	171	0.0015	78.4	TcoSat87	172	0.0017	75.6
TcoSat16 <sup>B</sup>	122	0.0011	74.6	TcoSat52	171	0.0015	70.2	TcoSat88	262	0.0015	67.2
TcoSat17 <sup>B</sup>	205	0.0044	74.1	TcoSat53	203	0.0015	71.4	TcoSat89	330	0.0015	73.3
TcoSat18 <sup>B</sup>	154	0.0019	74.7	TcoSat54	840	0.0165	67.3	TcoSat90	170	0.0014	68.8
TcoSat19 <sup>B</sup>	152	0.0044	77	TcoSat55	352	0.0049	69.9	TcoSat91	170	0.0014	78.2
TcoSat20 <sup>B</sup>	191	0.0027	73.8	TcoSat56	168	0.0030	70.8	TcoSat92*	136	0.0014	74.3
TcoSat21 <sup>B</sup>	303	0.0023	75.9	TcoSat57	575	0.0029	68.7	TcoSat93	153	0.0013	78.4
TcoSat22 <sup>B</sup>	122	0.0054	76.2	TcoSat58	248	0.0028	66.9	TcoSat94	192	0.0013	73.4
TcoSat23 <sup>B</sup>	181	0.0039	80.7	TcoSat59 <sup>E</sup>	162	0.0027	75.3	TcoSat95	263	0.0013	75.7
TcoSat24	196	0.0087	74.5	TcoSat60 <sup>E</sup>	164	0.0017	77.4	TcoSat96	93	0.0013	77.4
TcoSat25	121	0.0028	75.2	TcoSat61	83	0.0014	68.7	TcoSat97	415	0.0012	69.9
TcoSat26	105	0.0041	58.1	TcoSat62	83	0.0020	80.7	TcoSat98	345	0.0012	69.9
TcoSat27	302	0.0031	74.8	TcoSat63	150	0.0023	64.7	TcoSat99	181	0.0012	63.5

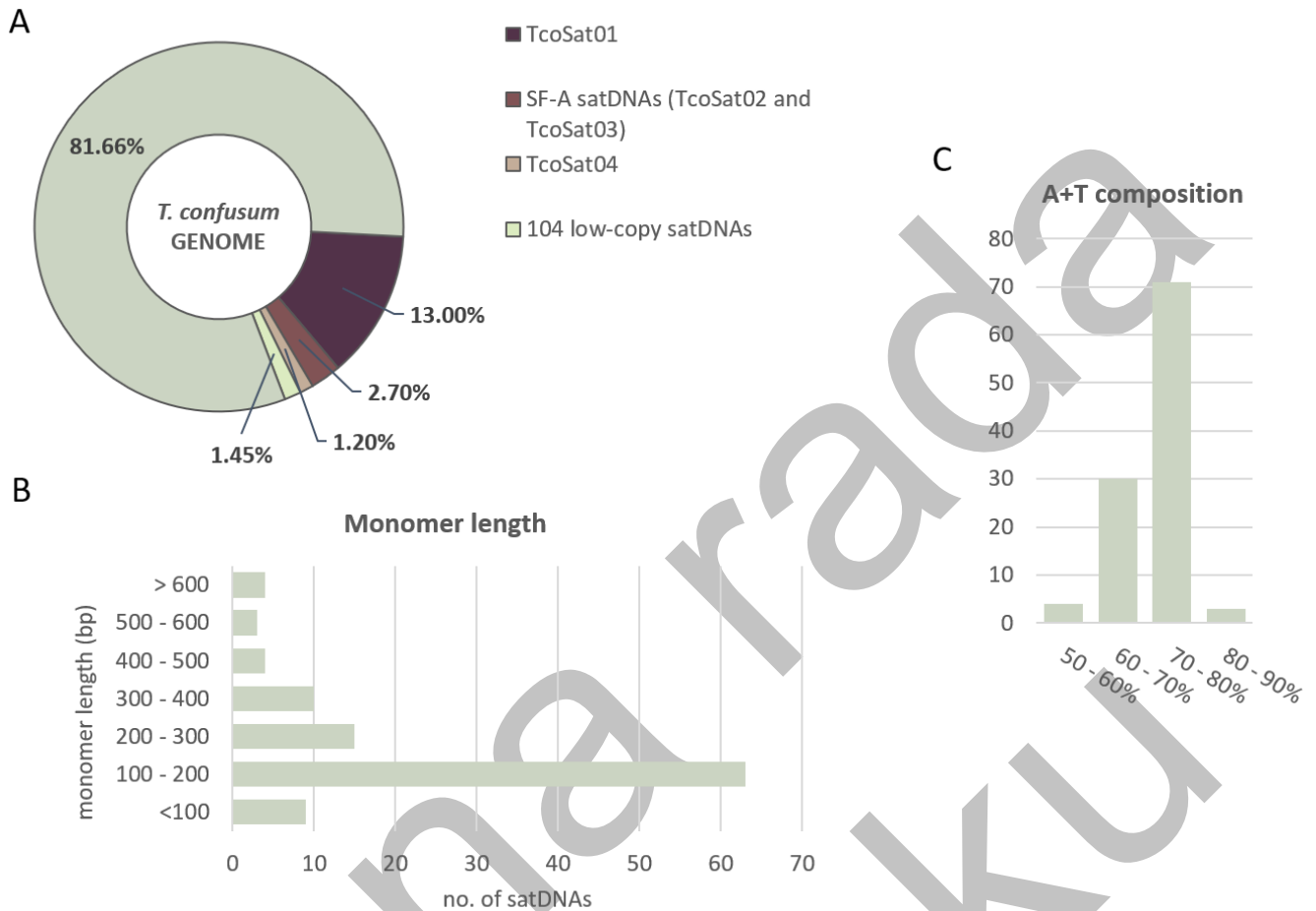


<b>TcoSat28</b>	161	0.0200	64	<b>TcoSat64</b>	154	0.0023	76	<b>TcoSat100</b>	60	0.0011	55
<b>TcoSat29</b>	101	0.0110	68.3	<b>TcoSat65</b>	211	0.0023	74.9	<b>TcoSat101</b>	102	0.0010	61.8
<b>TcoSat30</b>	682	0.6090	69.6	<b>TcoSat66</b>	93	0.0015	73.1	<b>TcoSat102</b>	146	0.0030	63.7
<b>TcoSat31</b>	265	0.0074	72.1	<b>TcoSat67<sup>F</sup></b>	220	0.0023	73.6	<b>TcoSat103</b>	371	0.0028	75.2
<b>TcoSat32</b>	168	0.0054	75.6	<b>TcoSat68<sup>F</sup></b>	195	0.0018	78.5	<b>TcoSat104</b>	183	0.0023	74.9
<b>TcoSat33</b>	189	0.0048	75.1	<b>TcoSat69</b>	277	0.0022	67.1	<b>TcoSat105</b>	171	0.0021	71.9
<b>TcoSat34</b>	153	0.0068	77.1	<b>TcoSat70</b>	234	0.0021	69.2	<b>TcoSat106</b>	174	0.0019	69
<b>TcoSat35<sup>C</sup></b>	62	0.0040	69.4	<b>TcoSat71</b>	170	0.0020	75.3	<b>TcoSat107</b>	387	0.0017	72.1
<b>TcoSat36<sup>C,*</sup></b>	130	0.0039	70.8	<b>TcoSat72</b>	159	0.0020	72.3	<b>TcoSat108</b>	184	0.0016	71.7

<sup>1</sup>SatDNA characterized previously in Plohl et al. 1993

The abundance of the major satDNA in the genome of *T. confusum* was experimentally estimated to be 40% (Plohl et al. 1993), but the number of the Illumina reads corresponding to the consensus sequence of TcoSat01 and the output of TAREAN analyses indicate that the estimate is 13% (Table 12, Figure 20A). Nevertheless, the major satDNA TcoSat01 represents the most abundant sequence in the genome of *T. confusum*. Only two of the newly characterized satDNAs, TcoSat02 and TcoSat04, are present in the genome with more than 1%, while the remaining 105 satDNA cumulatively make up 1.5% of the genome (Figure 20A). Altogether, the satellitome comprises 18.3% of the total *T. confusum* genomic content. Out of the 108 total satDNAs in the genome of *T. confusum*, 59 have shown similarity with repeats found in the RepBase, among which DNA transposons are the most common hit (Suppl. table 4).

The monomers of the *T. confusum* satDNAs vary in length, spanning from 60 to 840 bp. The majority of them are in the 100 – 200 bp range, with 176 bp being the median length of all of the monomers (Figure 20B). Regarding the nucleotide composition of the monomers, they are abundant in A+T nucleotides, with 71 of 108 satDNAs having the A+T composition in the range from 70-80% (Figure 20C).



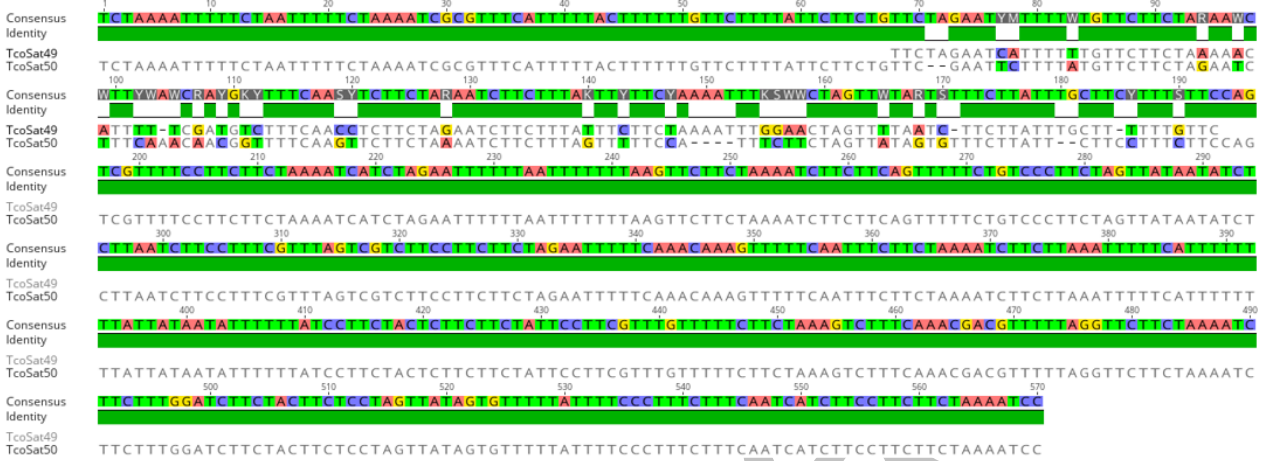
**Figure 20.** Share of satellitome in the total genomic content (A), monomer length distribution (B) and A+T composition (C) of consensus sequences of the 108 *Tribolium confusum* satellite DNAs.

Seven different superfamilies were observed among the satDNAs of the *T. confusum* (Table 12). Superfamily A consist of the satDNAs TcoSat02 and TcoSat03, whose consensus monomers show a pairwise identity of 76.1% (Table 12, Figure 21A). Superfamily B is comprised of nine different satDNAs TcoSat15/16/17/18/19/20/21/22/23 (Table 12). The average pairwise identity of satDNAs of superfamily B is 61.6%, with similarities between sequences spanning from 51.9% to 78.4% (Figure 21B). Furthermore, superfamily C consist of satDNAs TcoSat35/36/37, with their average pairwise identity being 75.1, ranging from 70.3% to 79% (Table 12, Figure 21C). Interestingly, TcoSat36 is a HOR based on the two subunits showing opposite orientation (Figure 22B), but still sharing the high pairwise identity of 84.4%. The next superfamilies with two mutually similar satDNAs are superfamily D, which is made up of TcoSat49 and TcoSat50 (Table 12) with pairwise identity of 69.0% (Figure 21D), superfamily E consisting of TcoSat59 and TcoSat60 (Table 12) that share a pairwise identity of 76.5% (Figure 21E), and superfamily F with TcoSat67 and TcoSat68 (Table 12) sharing pairwise identity of

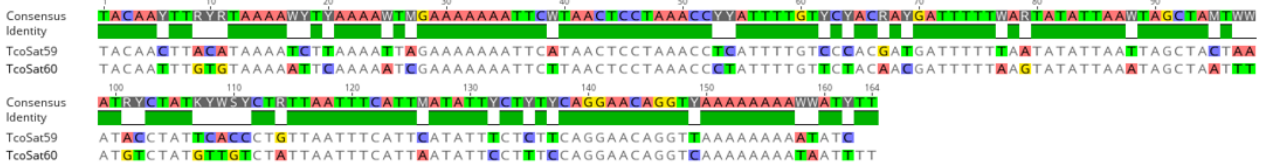




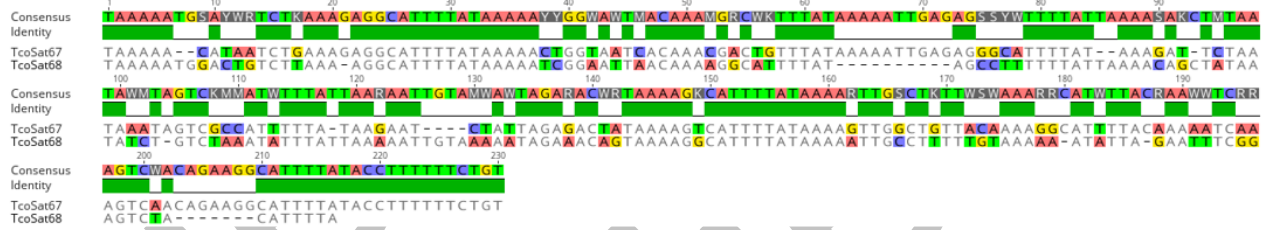
**D Superfamily D – TcoSat49/50, pairwise identity = 69.0%**



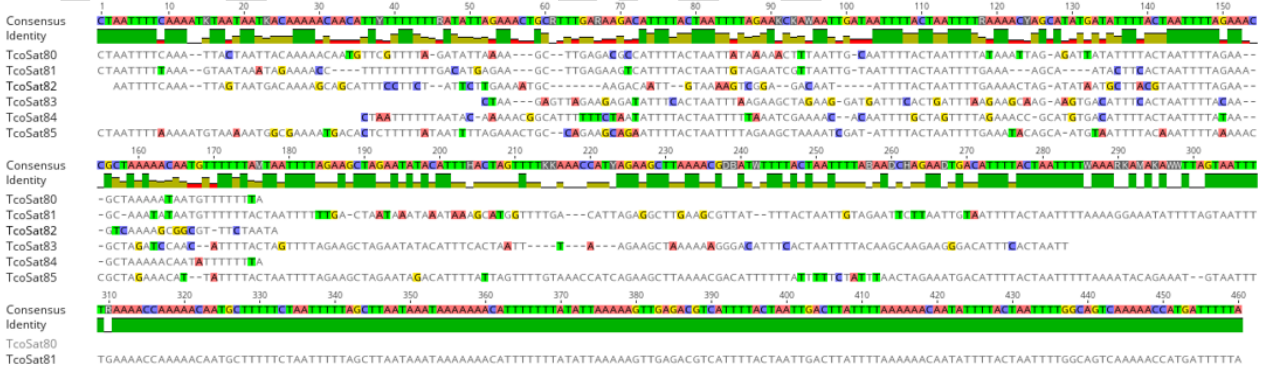
**E Superfamily E – TcoSat59/60, pairwise identity = 76.5%**



**F Superfamily F – TcoSat67/68, pairwise identity = 62.5%**



**G Superfamily G – TcoSat80/81/82/83/84/85**

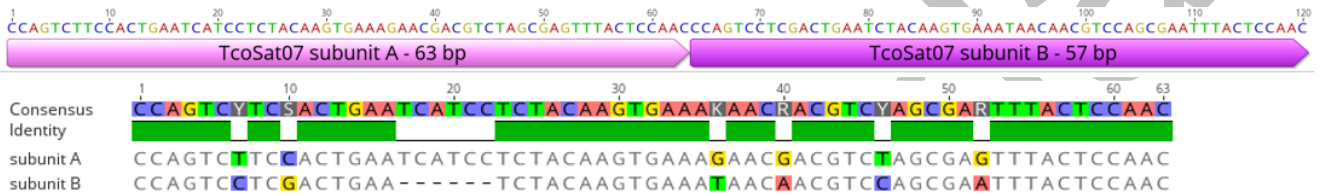


	TcoSat80	TcoSat81	TcoSat82	TcoSat83	TcoSat84	TcoSat85
TcoSat80	-	74.56%	60.12%	60.47%	66.67%	64.97%
TcoSat81	74.56%	-	55.81%	54.95%	61.27%	58.96%
TcoSat82	60.12%	55.81%	-	54.34%	52.52%	56.32%
TcoSat83	60.47%	54.95%	54.34%	-	63.83%	65.51%
TcoSat84	66.67%	61.27%	52.52%	63.83%	-	65.73%
TcoSat85	64.97%	58.96%	56.32%	66.51%	65.73%	-

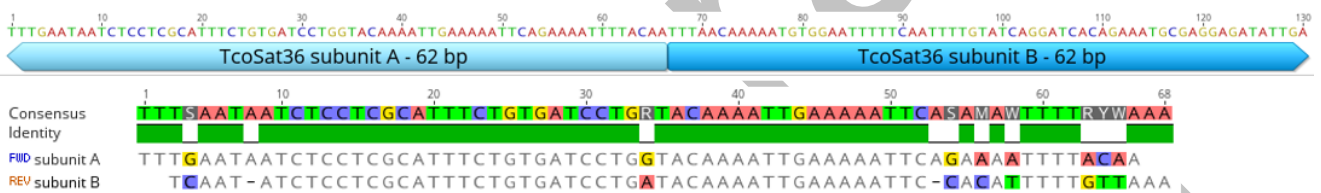
**Figure 21.** Geneious alignments and distance matrices of *Tribolium confusum* satellite DNAs belonging to the superfamily A (A), superfamily B (B), superfamily C (C), superfamily D (D), superfamily E (E), superfamily F (F), and superfamily G (G).

Except for the TcoSat36, two other *T. confusum* satDNAs have HOR monomers (Table 12). Both satDNAs, TcoSat07 and TcoSat92 are of dimeric structure, with subunits being 81.0% and 81.2% similar, respectively (Figure 22A and 22C).

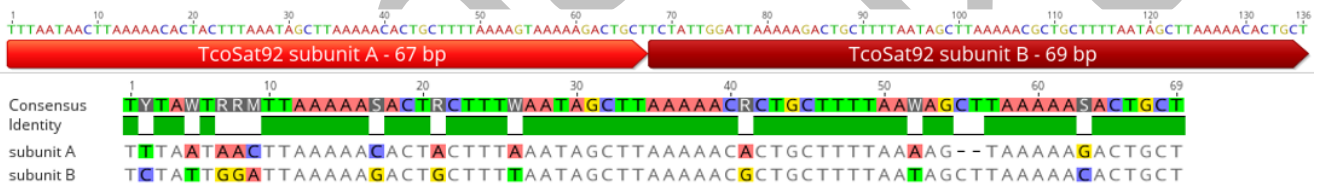
**A TcoSat07 – pairwise identity = 81.0%**



**B TcoSat36 – pairwise identity = 84.4%**



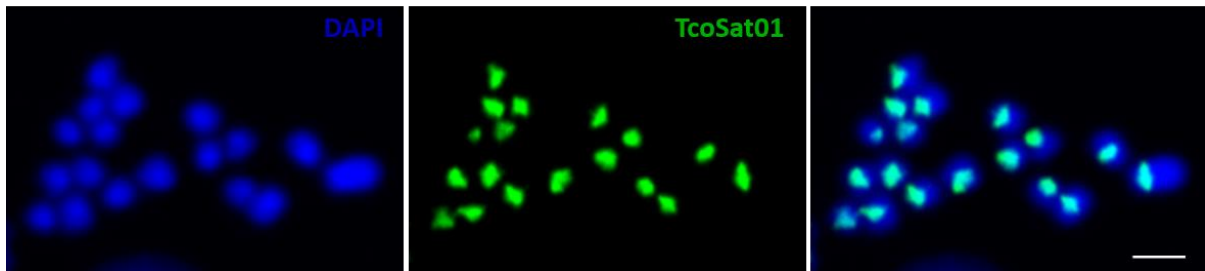
**C TcoSat92 – pairwise identity = 81.2%**



**Figure 22.** Schematic representation of the higher-order repeat structures of satellite DNAs TcoSat07 (A), TcoSat36 (B), and TcoSat92 (C). For each satDNA, Geneious alignments of the subunits and subunits' pairwise identities are shown.

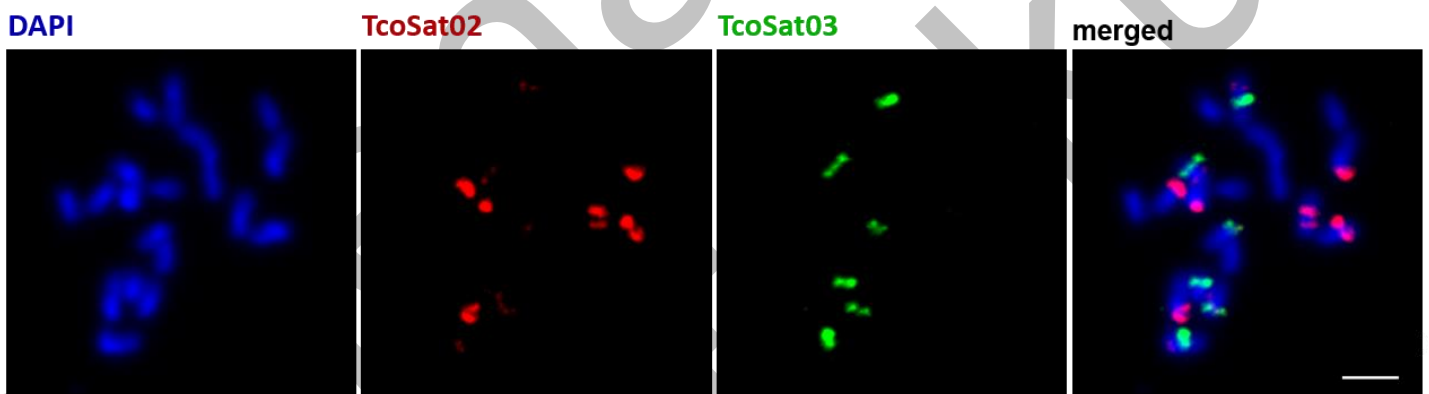
**3.3.3. Chromosome localization of the *T. confusum* satellite DNAs**

FISH experiments were performed to localize the most prominent satDNAs of *T. confusum* on the chromosomes. Major satDNA TcoSat01 is present in large heterochromatic blocks on all of the chromosomes of the complement (2n=18) (Figure 23).



**Figure 23.** Chromosomes of male *Tribolium confusum* specimen stained by DAPI and *in situ* hybridized with a FITC-labelled probe specific for TcoSat01. Scale bar represents 3  $\mu$ m.

Due to the nucleotide sequence similarities between the satDNAs TcoSat02 and TcoSat03 (superfamily A), a double FISH was performed to determine their chromosomal position simultaneously. As shown in Figure 24, both satellites are present on approximately three pairs of the chromosomes, but they do not colocalize on the same chromosomes. The signals appear to be located next to the large heterochromatic blocks, therefore it can be concluded that the satDNAs in question are predominately located in pericentromeric regions.

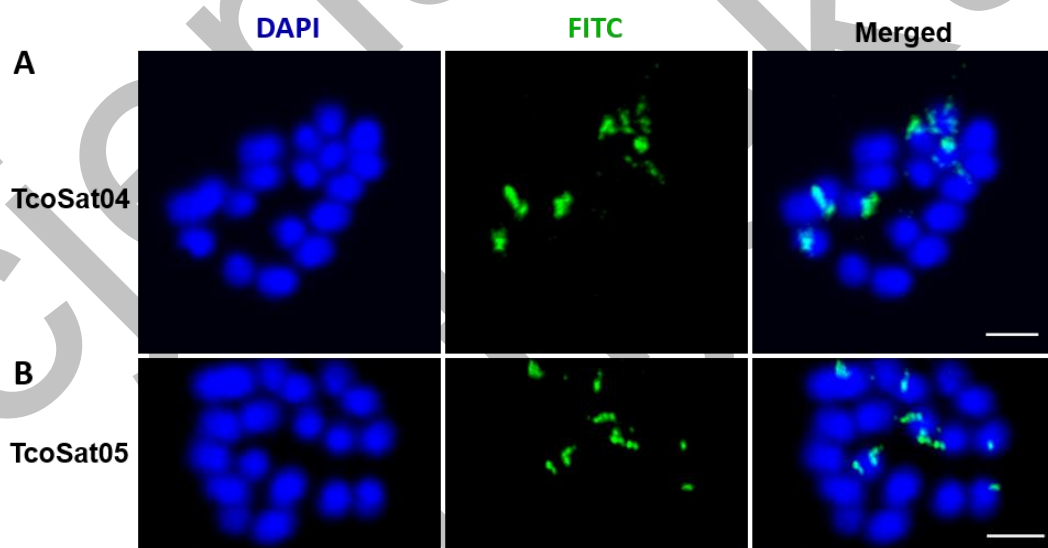


**Figure 24.** *Tribolium confusum* chromosome spreads from male gonads, stained by DAPI (blue) and hybridized *in situ* with Cy3-labelled probe specific for TcoSat02 (red fluorescence) and FITC-labelled probe specific for TcoSat01 (green fluorescence). Scale bar represents 3  $\mu$ m.

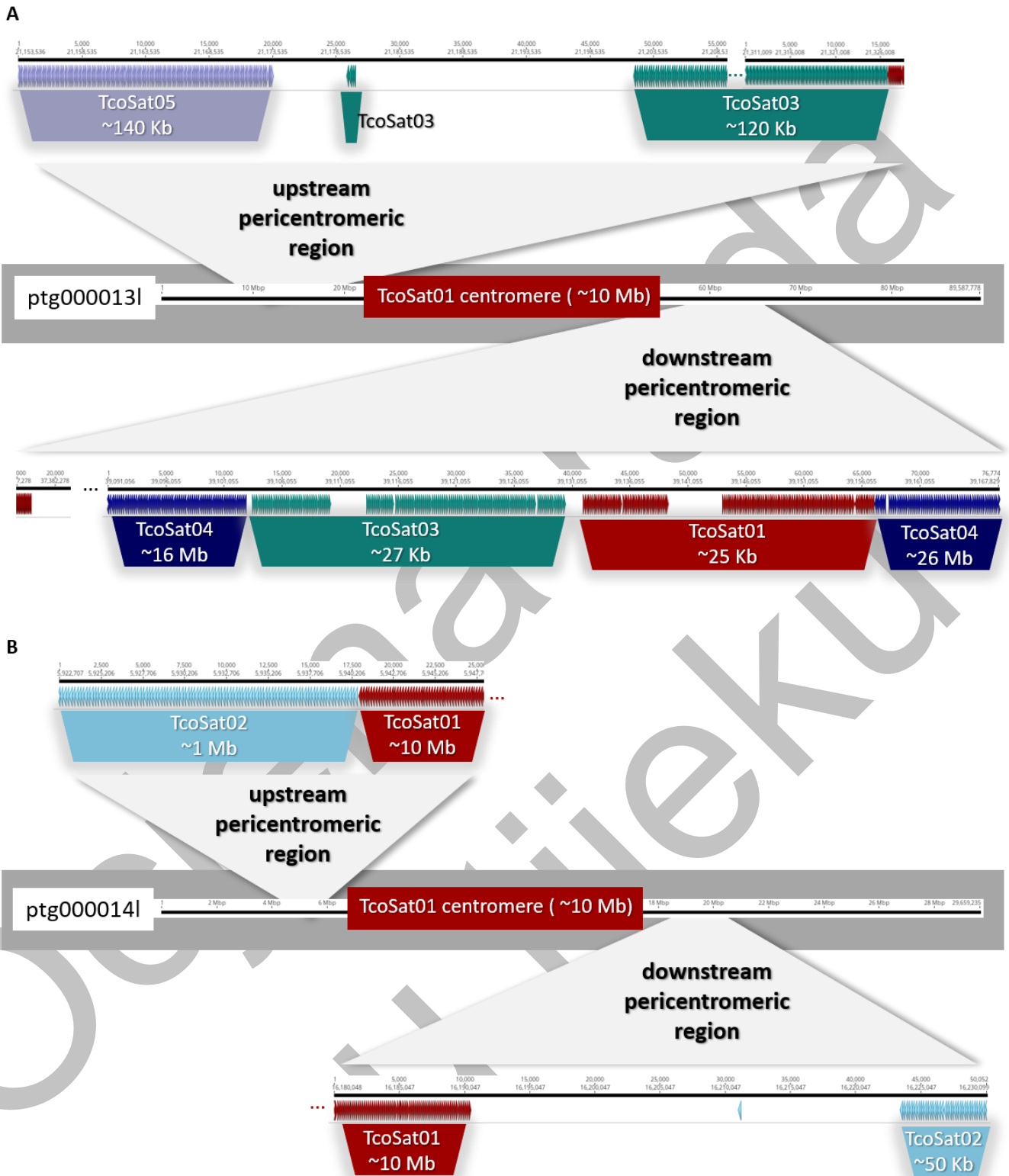
FISH experiments were also performed for the six low-copy-number satDNAs, TcoSat04, TcoSat05, TcoSat06, TcoSat07, TcoSat08, and TcoSat09.

TcoSat04 is located on half of the chromosomes of the complement with the signals that are more dispersed than those of the major satDNA TcoSat01 (Figure 25A). The signals of the satDNA TcoSat05 are similar in appearance to those of TcoSat04, also being present on half of the chromosomes (Figure 25B). The abundance of the signals is in accordance to their higher genomic percentage in comparison to other low-copy satDNAs. Furthermore, due to the localization of the signals, it can be concluded

that satDNAs TcoSat04 and TbrSat05 are also positioned in pericentromeric regions. Considering that the heterochromatic blocks are primarily made of the major satDNA TcoSat1, cytogenetic localization of satDNAs TcoSat02-03-04-05 in pericentromeric regions was additionally explored *in silico* by analyzing the preliminary assembly of *T. confusum*. The major satDNA TcoSat01 mapped on some of the longest contigs of the preliminary assembly in arrays of more than 10 Mb, making them likely candidates for centromeric regions. Flanking regions surrounding centromeric TcoSat01 arrays of the contigs ptg000013l and ptg000014l were further explored (Figure 26). The upstream region of the ptg000013l centromere contained arrays of the TcoSat05 (~140 Kb) and TcoSat03 (~120 Kb) satDNAs, while the downstream region was occupied by two arrays of TcoSat04 (~16 Mb + ~26 Mb) and an array of TcoSat03 (~25 Kb) (Figure 26A). Regarding the ptg000014l, both regions surrounding TcoSat01 array consist of TcoSat02 arrays (upstream ~1 Mb, downstream (~50 Kb) (Figure 26B). *In silico* analyses therefore confirmed the position of satDNAs TcoSat02-03-04-05 in pericentromeric regions of *T. confusum* chromosomes. Additionally, no arrays of TcoSat02 and TcoSat03 were found mapped together in pericentromeric regions, in accordance to the double FISH experiments (Figure 24).



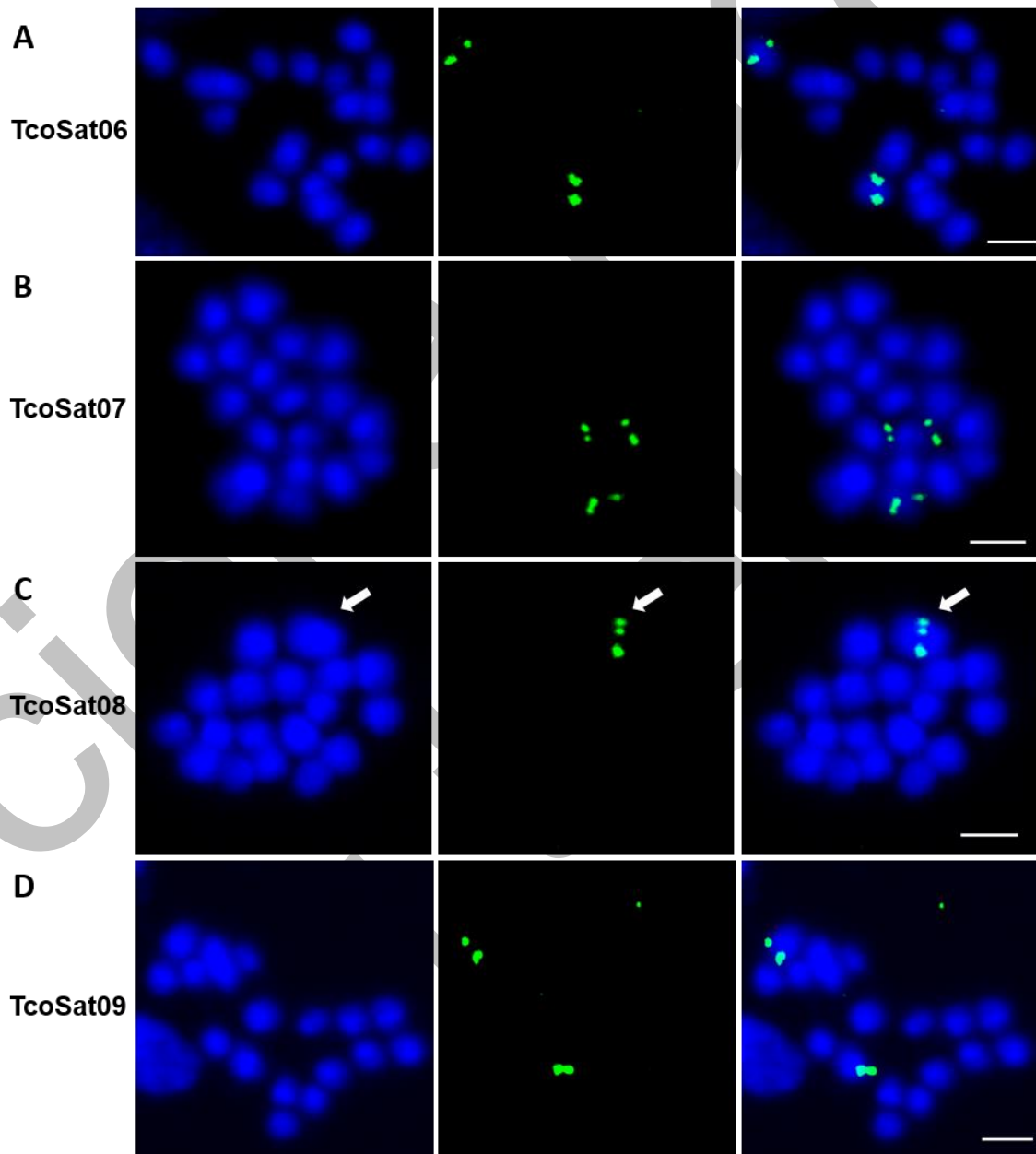
**Figure 25.** *Tribolium confusum* male chromosome spreads stained by DAPI (blue) and hybridized with FITC-labelled probes (green) specific for the satellite DNAs TcoSat04 (A), TcoSat05 (B). Scale bar represents 3  $\mu$ m.



**Figure 26.** Schematic representation of (peri)centromeric organization of *Tribolium confusum* chromosomes. A) Long range organization of contig ptg0000131 with annotated TcoSat01 centromeric region (red box) and pericentromeric regions containing satellite DNAs TcoSat03 (turquoise box), TcoSat04 (dark blue box) and TcoSat05 (purple box). B) Long range organization of contig ptg0000141 with annotated TcoSat01 centromeric region (red box) and pericentromeric region containing satellite DNA TcoSat05 (light blue box).

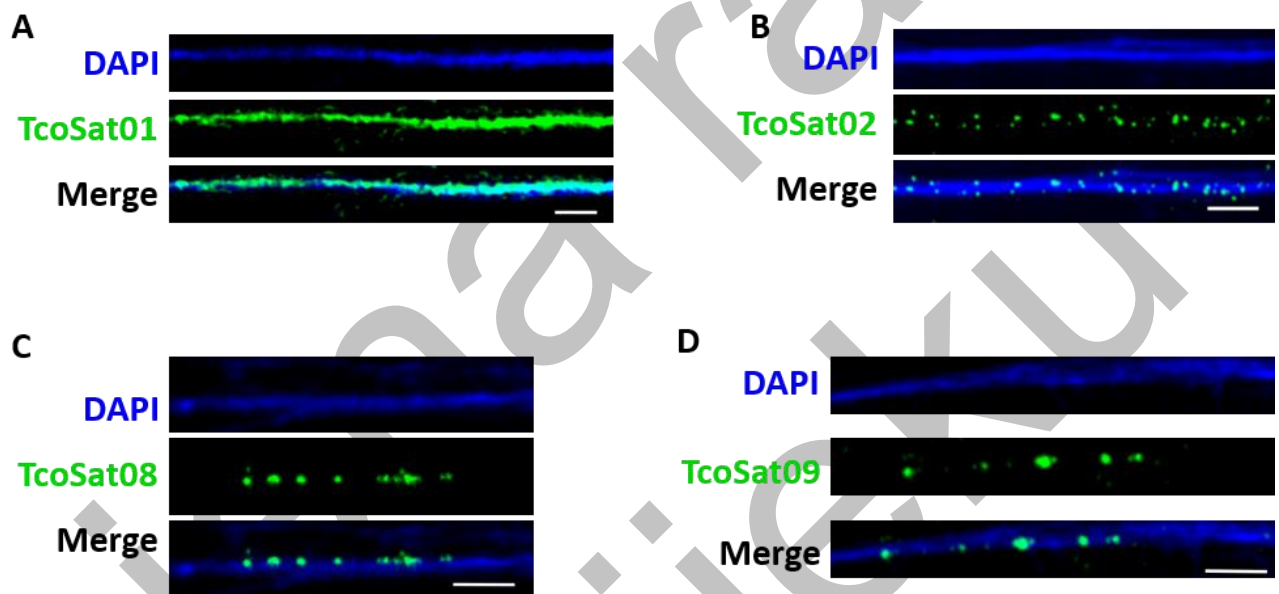


TcoSat06 (Figure 27A), as well as TcoSat09 (Figure 27D), showed signal on only one pair of chromosomes. TcoSat07 is located on two chromosome pairs (Figure 27B). TcoSat08 was detected on only one chromosome. The mentioned chromosome is in fact the biggest chromosome of the complement, so it can be assumed that the said chromosome is the sex neo-X chromosome (Figure 27C).



**Figure 27.** *Tribolium confusum* male chromosome spreads stained by DAPI (blue) and hybridized with FITC-labelled probes (green) specific for the satellite DNAs TcoSat06 (A), TcoSat07 (B), TcoSat08 (C) and TcoSat09 (D). An arrow shows the sex chromosome neo-X. Scale bar represents 3  $\mu\text{m}$ .

To compare the long-range organization of abundant satDNAs and low-copy-number satDNAs, TcoSat01, TcoSat02, TcoSat08 and TcoSat09 were chosen for the FISH experiments on the extended chromosome fibers. The major satDNA TcoSat01 showed long continuous organization, dominating the whole length of the chromosome fibers (Figure 28A). TcoSat02 is the moderate-copy-number satDNA, and its more scattered signals (Figure 28B) reflect lower abundance compared to the major satDNA TcoSat01. As the representatives of low-copy satDNAs, TcoSat08 and TcoSat09 show a similar pattern of organization, made up of shorter arrays with less signals that are scattered on the chromosome fibers (Figures 28C and 28D).



**Figure 28.** Extended DNA fibers of *Tribolium confusum* stained by DAPI (blue) and hybridized with FITC-labelled probes (green) specific for the satellite DNAs TcoSat01 (A), TcoSat02 (B), TcoSat08 (C), TcoSat09 (D). Scale bar represents 3  $\mu\text{m}$ .

### 3.4. Satellitome of the species *Tribolium brevicornis*

#### 3.4.1. Detection of satellite DNAs in *T. brevicornis* genome

The satDNAs of the North American flour beetle *T. brevicornis* were determined by whole genome sequences obtained using the Illumina platform. The sequencing provided 35 262 904 paired-end reads that were 151 bp long (Table 6). To characterize the maximum amount of satDNAs, six different TAREAN runs were performed (Table 13). Analyses TB1 – TB3 were performed with random subsets of the total Illumina reads, but the major satDNA TBREV (Mravinac et al. 2005) clogged analysis TB3, which was run with the highest coverage. Therefore, subsequent TAREAN analyses were performed with the subset of 29 987 536 paired-end reads lacking the major satDNA.

**Table 13.** Summary of the results of six different TAREAN analyses performed for the North American flour beetle *Tribolium brevicornis*.

TAREAN clustering analysis	TB1	TB2	TB3	TB4*	TB5*	TB6*
No. of input reads	185,000	460,000	920,000	920,000	1,390,000	1,850,000
No. of analyzed reads	183,316	455,856	885,412	911,482	1,377,316	1,833,126
Genome coverage	0.1x	~0.25x	~0.5x	~0.5x	~0.75x	~1x
Proportion of reads in top clusters	33%	40%	45%	38%	41%	43%
No. of reads in clusters	87,998 (47.57%)	253,773 (55.67%)	550,955 (62.23%)	532,159 (58.38%)	886,585 (64.37%)	1,264,602 (68.99%)
No. of clusters	9,615	25,102	56,319	71,806	123,543	180,242
No. of singlets	95,318	202,083	334,457	379,323	490,731	568,524
No. of satellites	16	24	48	60	111	148
No. of high putative satellites	3	7	15	14	28	37
No. of low putative satellites	13	17	33	46	83	111

\*analyses performed with the subset of reads missing the major satDNA TBREV (Mravinac et al. 2005)

A preliminary assembly of *T. brevicornis* was assembled using PacBio HiFi reads (unpublished results). It was used to determine the tandem organization of potentially novel satDNAs found by TAREAN. The consensus sequences were annotated to the preliminary assembly applying the 70% similarity criterion, and the sequences that showed tandem organization of 5 or more monomers were characterized as satDNAs.



### 3.4.2. Analysis of the satellitome of *T. brevicornis*

The 165 new satDNAs were found in the genome of the North American flour beetle *T. brevicornis* using *in silico* methods. Thus, the satellitome of *T. brevicornis* comprises a total of 166 satDNAs, including the previously characterized major satDNA TBREV. For additional clarity, satDNA TBREV is referred to as TbrSat01 in this work (Table 14). Consensus sequences of the *T. brevicornis* satellitome can be found in Supplementary table 1.

**Table 14.** Satellitome of the North American flour beetle *Tribolium brevicornis*. The table contains the main characteristics of satellite DNAs: repeat unit (monomer) lengths, the genome proportion, the A+T base composition of the individual satellite DNAs. The satellite DNAs with higher-order-repeats (HORs) are marked with asterisk (\*), while the satellite DNAs that belong to the same superfamily are color-coded and labelled with superscripts A, B, C, D, E and F.

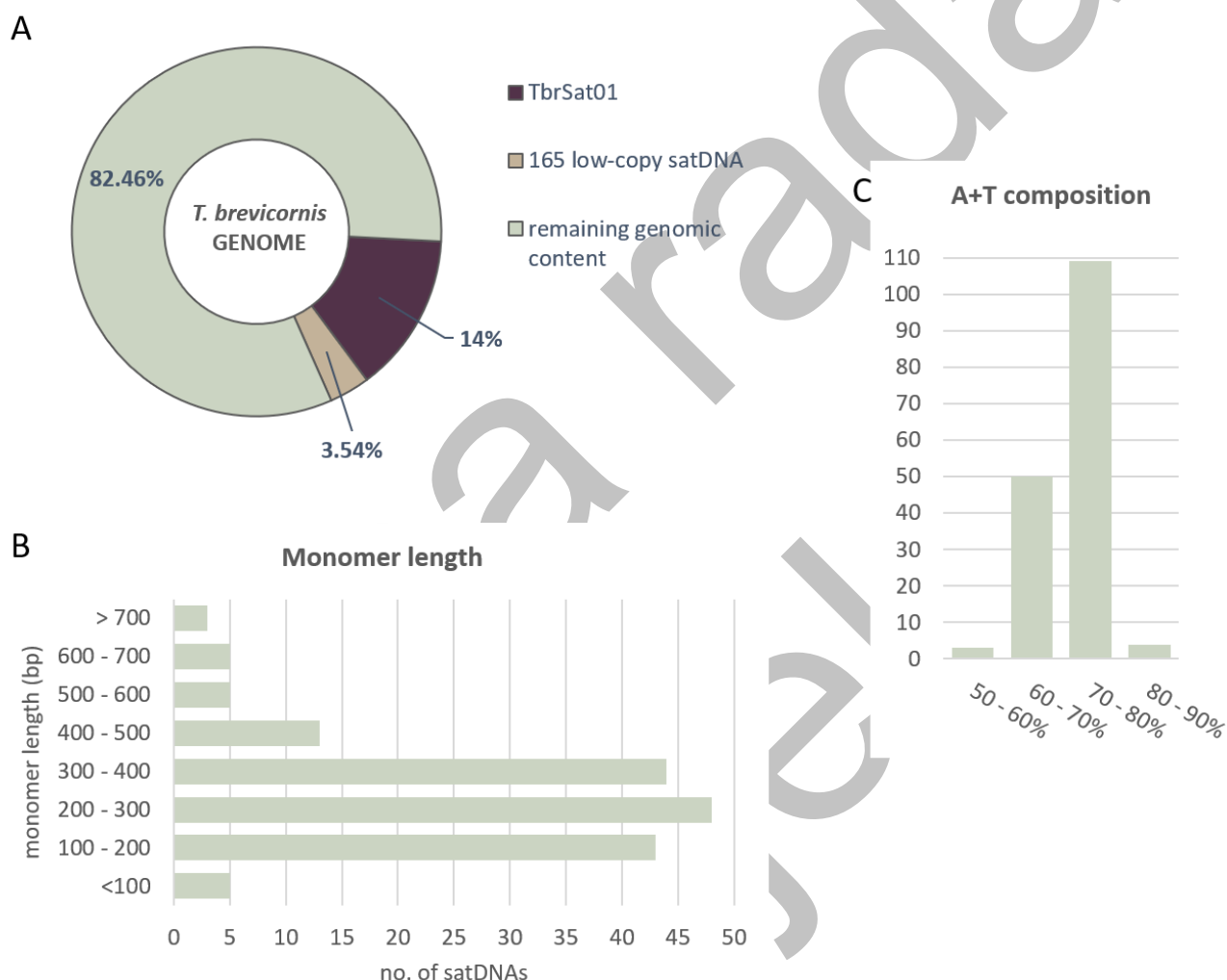
satDNA	length (bp)	% genome	AT%	satDNA	length (bp)	% genome	AT%	satDNA	length (bp)	% genome	AT%
TbrSat01*	1061	14	69.2	TbrSat57	337	0.0029	70.9	TbrSat112	388	0.0013	69.8
TbrSat02 <sup>A</sup>	165	0.37	78.8	TbrSat58	173	0.0024	74.0	TbrSat113	91	0.0013	78.0
TbrSat03 <sup>A</sup>	165	0.076	76.4	TbrSat59	287	0.0016	73.5	TbrSat114	152	0.0013	69.1
TbrSat04 <sup>A</sup>	172	0.0011	76.7	TbrSat60	304	0.0026	71.4	TbrSat115	506	0.0013	76.1
TbrSat05	102	0.2408	60.8	TbrSat61	335	0.0026	72.2	TbrSat116	288	0.0013	76.0
TbrSat06 <sup>B</sup>	166	0.0327	69.9	TbrSat62	423	0.0026	72.3	TbrSat117	153	0.0013	71.2
TbrSat07 <sup>B</sup>	169	0.0021	70.4	TbrSat63	249	0.0023	75.5	TbrSat118	611	0.0026	78.9
TbrSat08 <sup>B</sup>	163	0.0027	67.5	TbrSat64	163	0.0022	71.2	TbrSat119	547	0.0024	76.2
TbrSat09 <sup>B,*</sup>	330	0.0015	65.8	TbrSat65	456	0.0022	77.2	TbrSat120 <sup>F</sup>	164	0.0022	73.2
TbrSat10	491	0.0241	67	TbrSat66	307	0.0019	71.3	TbrSat121 <sup>F</sup>	301	0.0011	71.1
TbrSat11 <sup>C</sup>	150	0.0220	65.3	TbrSat67	243	0.0019	73.3	TbrSat122	339	0.0022	68.4
TbrSat12 <sup>C</sup>	171	0.0055	65.5	TbrSat68 <sup>D</sup>	173	0.0024	76.3	TbrSat123*	224	0.0020	75.4
TbrSat13	175	0.0180	68.6	TbrSat69 <sup>D</sup>	323	0.0022	71.2	TbrSat124	307	0.0018	75.2
TbrSat14	216	0.0210	70.4	TbrSat70 <sup>E</sup>	694	0.0112	65.6	TbrSat125	490	0.0018	76.5
TbrSat15	249	1.2500	71.5	TbrSat71 <sup>E</sup>	698	0.0035	66.0	TbrSat126	383	0.0017	75.2
TbrSat16*	52	0.0361	53.8	TbrSat72 <sup>E</sup>	634	0.0035	66.1	TbrSat127	271	0.0017	73.8
TbrSat17	174	0.4042	74.7	TbrSat73	342	0.0032	81.3	TbrSat128	305	0.0017	66.9
TbrSat18	847	0.5500	67.4	TbrSat74	311	0.0031	70.4	TbrSat129	144	0.0016	81.2
TbrSat19	143	0.0560	65	TbrSat75	476	0.0031	72.1	TbrSat130	515	0.0016	72.2
TbrSat20	133	0.0460	69.2	TbrSat76	411	0.0028	74.7	TbrSat131	466	0.0016	74.2
TbrSat21	155	0.0048	63.2	TbrSat77	233	0.0027	74.7	TbrSat132	306	0.0016	66.0
TbrSat22	147	0.0053	63.9	TbrSat78	283	0.0026	76.0	TbrSat133	335	0.0015	74.0
TbrSat23	120	0.0077	64.2	TbrSat79	295	0.0026	81.4	TbrSat134	323	0.0015	73.4
TbrSat24	105	0.0028	70.5	TbrSat80*	345	0.0024	71.6	TbrSat135	558	0.0015	71.9
TbrSat25	75	0.0072	53.3	TbrSat81	442	0.0024	70.4	TbrSat136	260	0.0015	76.2
TbrSat26	164	0.0210	67.1	TbrSat82	204	0.0022	72.5	TbrSat137	257	0.0015	75.9
TbrSat27	246	0.0068	60.2	TbrSat83	175	0.0022	73.1	TbrSat138	344	0.0014	71.5

<b>TbrSat28</b>	327	0.0066	74.3	<b>TbrSat84</b>	248	0.0021	76.2	<b>TbrSat139</b>	345	0.0014	72.8
<b>TbrSat29</b>	335	0.0065	75.5	<b>TbrSat85</b>	461	0.0021	74.6	<b>TbrSat140</b>	251	0.0013	72.1
<b>TbrSat30</b>	316	0.0066	74.7	<b>TbrSat86</b>	282	0.0021	74.1	<b>TbrSat141</b>	194	0.0013	72.2
<b>TbrSat31</b>	338	0.0044	72.2	<b>TbrSat87</b>	445	0.0021	70.1	<b>TbrSat142</b>	677	0.0013	71.6
<b>TbrSat32</b>	350	0.0075	67.7	<b>TbrSat88</b>	285	0.0020	75.8	<b>TbrSat143</b>	143	0.0013	79.7
<b>TbrSat33</b>	489	0.0070	68.9	<b>TbrSat89</b>	325	0.0020	72.9	<b>TbrSat144</b>	163	0.0012	77.9
<b>TbrSat34</b>	355	0.0054	72.1	<b>TbrSat90</b>	224	0.0019	72.3	<b>TbrSat145</b>	341	0.0012	70.7
<b>TbrSat35</b>	317	0.0038	73.8	<b>TbrSat91</b>	57	0.0019	50.9	<b>TbrSat146</b>	159	0.0012	76.1
<b>TbrSat36</b>	236	0.0038	69.5	<b>TbrSat92</b>	235	0.0019	66.4	<b>TbrSat147</b>	185	0.0012	70.3
<b>TbrSat37</b>	209	0.0036	70.3	<b>TbrSat93</b>	252	0.0019	63.5	<b>TbrSat148</b>	212	0.0012	68.4
<b>TbrSat38</b>	287	0.0035	66.6	<b>TbrSat94</b>	293	0.0018	69.6	<b>TbrSat149</b>	301	0.0012	66.8
<b>TbrSat39</b>	155	0.0034	79.4	<b>TbrSat95</b>	337	0.0016	75.1	<b>TbrSat150</b>	134	0.0012	65.7
<b>TbrSat40</b>	305	0.0030	75.1	<b>TbrSat96</b>	60	0.0017	60.0	<b>TbrSat151</b>	134	0.0011	70.9
<b>TbrSat41</b>	170	0.0029	72.9	<b>TbrSat97</b>	922	0.0172	64.6	<b>TbrSat152</b>	269	0.0011	68.8
<b>TbrSat42</b>	245	0.0028	72.7	<b>TbrSat98</b>	324	0.0017	70.1	<b>TbrSat153</b>	165	0.0011	71.5
<b>TbrSat43</b>	315	0.0028	70.2	<b>TbrSat99</b>	224	0.0017	77.7	<b>TbrSat154</b>	327	0.0010	77.1
<b>TbrSat44*</b>	226	0.0027	72.1	<b>TbrSat100</b>	508	0.0016	74.6	<b>TbrSat155</b>	266	0.0010	69.5
<b>TbrSat45</b>	354	0.0027	72.6	<b>TbrSat101</b>	289	0.0016	73.7	<b>TbrSat156</b>	165	0.0009	77.6
<b>TbrSat46</b>	235	0.0027	68.5	<b>TbrSat102</b>	172	0.0015	69.8	<b>TbrSat157</b>	300	0.0009	72.3
<b>TbrSat47</b>	212	0.0027	69.8	<b>TbrSat103</b>	177	0.0015	71.2	<b>TbrSat158</b>	331	0.0009	68.0
<b>TbrSat48</b>	289	0.0027	71.6	<b>TbrSat104*</b>	336	0.0015	69.0	<b>TbrSat159</b>	215	0.0009	76.3
<b>TbrSat49</b>	298	0.0026	69.8	<b>TbrSat105</b>	320	0.0015	71.9	<b>TbrSat160</b>	187	0.0009	70.1
<b>TbrSat50</b>	209	0.0026	70.8	<b>TbrSat106</b>	267	0.0015	68.2	<b>TbrSat161</b>	257	0.0009	73.5
<b>TbrSat51</b>	404	0.0026	70.5	<b>TbrSat107</b>	327	0.0014	76.1	<b>TbrSat162</b>	235	0.0009	74.0
<b>TbrSat52</b>	243	0.0025	70.4	<b>TbrSat108</b>	303	0.0014	74.3	<b>TbrSat163</b>	282	0.0009	82.6
<b>TbrSat53</b>	269	0.0023	72.9	<b>TbrSat109</b>	299	0.0014	68.9	<b>TbrSat164</b>	180	0.0009	64.4
<b>TbrSat54</b>	335	0.0040	72.2	<b>TbrSat110</b>	332	0.0013	71.7	<b>TbrSat165</b>	282	0.0009	79.8
<b>TbrSat55</b>	147	0.0035	70.1	<b>TbrSat111</b>	421	0.0013	77.0	<b>TbrSat166</b>	295	0.0009	63.7
<b>TbrSat56</b>	143	0.0033	68.5								

<sup>1</sup>SatDNA TBREV previously characterized in Mravinac et al. 2005

TAREAN analyses indicate that the major satDNA TbrSat01 makes up 14% of the genome. Even though that number differs from the experimental estimation of 21% (Mravinac et al. 2005), the *in silico* analyses also show that it is the most dominant satDNA in the *T. brevicornis* genome. In contrast, 165 newly defined satDNAs are mostly low-copy-number satDNAs, with genome abundance being less than 0.4% each. In total, the 165 low-copy-number satDNAs account for 3.5% of the genomic content of *T. brevicornis* (Mravinac et al. 2005). Overall, the satellitome makes up 17.5% of the total genome of *T. brevicornis* (Figure 29A). Even though the satDNAs of *T. brevicornis* have a wide range of monomer lengths spanning between 52 and 1061 bp, the majority of satDNAs, 135 of them, are between 100 and 400 bp long. The median length of satDNA monomers of *T. brevicornis* satDNAs is 282 bp (Figure 29B). The characterized satDNAs are A+T rich, with only 3 satDNAs having an A+T

composition of less than 60%. The A+T composition of the majority of the satDNAs (109 of 165) is between 70% and 80% (Figure 29C). By comparing the satellitome to RepBase, it was found that 113 of satDNAs have similarities with already known repetitive elements, mainly DNA transposons (Suppl. table 5).

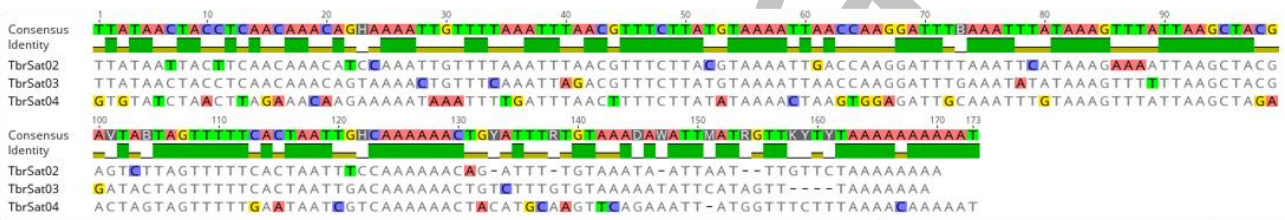


**Figure 29.** Share of the satellitome in the total genomic content (A), monomer length distribution (B) and A+T composition (C) of consensus sequences of the 167 *Tribolium brevicornis* satellite DNAs.

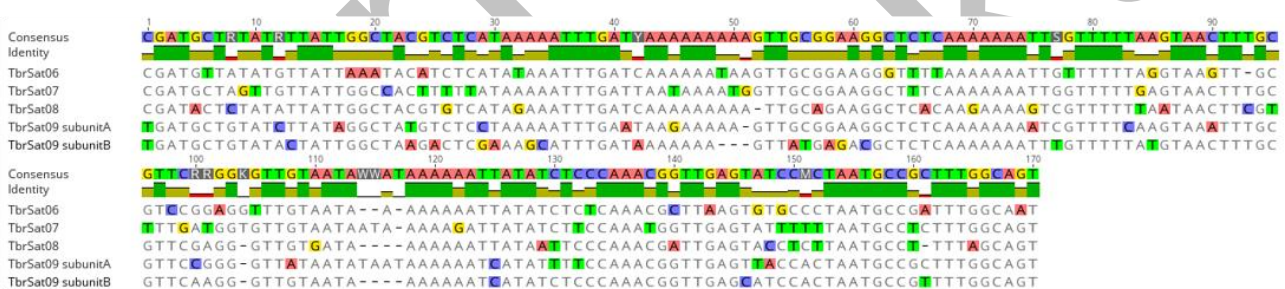
Six different superfamilies of satDNAs were detected in the satellitome of *T. brevicornis* (Table 14). The first is superfamily A, which consists of the satDNAs TbrSat02-04. The consensus sequences of their monomers show an average identity of 68.5%, while the monomers of TbrSat02 and TbrSat03 are even more similar with a percentage of 78.1% (Table 14, Figure 30A). Superfamily B is made of four different satDNAs (TbrSat06-09) whose monomers have an average pairwise identity of 72.8% with a span from 70.2% to 75.4% (Table 14, Figure 30B). In addition, the monomeric unit of TbrSat09

is a dimeric HOR, whose subunits, mutually similar 77.9% (Figure 31A), share 71.8-74.0% similarity with the remaining satDNAs of superfamily B (Figure 30B). The next superfamilies are superfamily C, consisting of TbrSat11 and TbrSat12 (Table 14) with a pairwise identity of 69.9% (Figure 30C) and superfamily D, consisting of TbrSat68 and TbrSat69 (Table 14), which have a pairwise identity of 73.6% (Figure 30D). Superfamily E is comprised of the three satDNAs, TbrSat70/71/72, whose average pairwise identity is 65.8%, but spans from 64.1% to 67.4% (Table 14, Figure 30E). SatDNAs TbrSat120 and TbrSat121 are part of superfamily F and share pairwise identity of 70.7% (Table 14, Figure 30F).

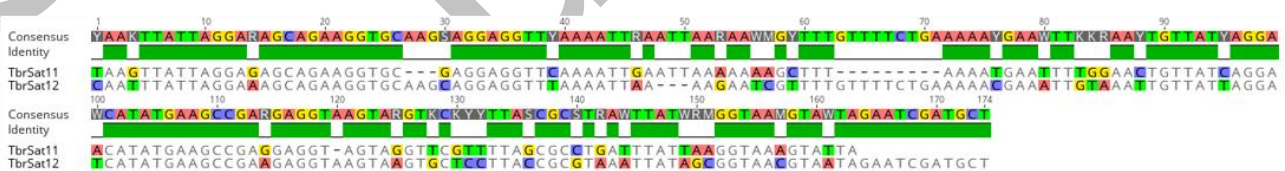
### A Superfamily A – TbrSat02/03/04



### B Superfamily B – TbrSat06/07/08/09 (subunits A and B)



### C Superfamily C – TbrSat11/12, pairwise identity = 69.9%

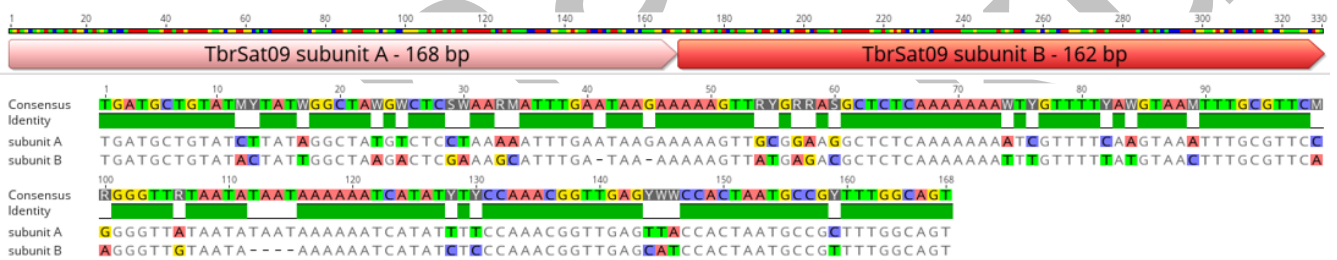




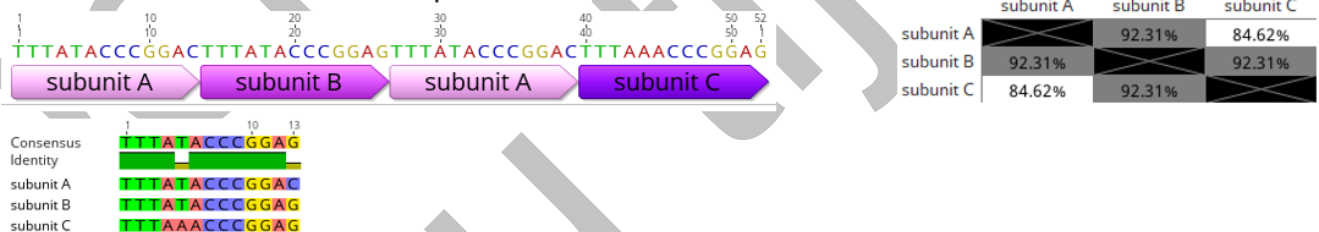


The monomers of several *T. brevicornis* satDNAs have an HOR structure. Apart from the already mentioned TbrSat09 (Figure 31A, Figure 30B), there are five other satDNAs with HOR monomer units. TbrSat16 has a repeat unit which comprises 13 bp subunits that repeat four times in a monomer, which is why it can be considered a tetrameric HOR (Figure 31B). The subunits share an average pairwise identity of 91.0% with two of them (the first and third subunits) being identical (Figure 31B). Although the short length of the subunits could mean that it is fact a monomer unit of a minisatellite, the long-range organization of TbrSat16 with the arrays of up to 120 kb in length annotated in the preliminary assembly indicates that TbrSat16 is satDNA. The monomer of satDNA TbrSat44 has a trimeric HOR structure, with the central subunit having a truncated form. The three subunits share an average pairwise identity of 67.6% (62.5% – 77.5%) (Figure 31C). The monomers of the satDNAs TbrSat80, TbrSat104 and TbrSat123 have a structure of a dimeric HOR. The subunits of the satDNA TbrSat80 show a pairwise identity of 82.8% (Figure 31D), those of TbrSat104 share a pairwise identity of 74.8% (Figure 31E), while the subunits of TbrSat123 have a pairwise identity of 77.6% (Figure 31F).

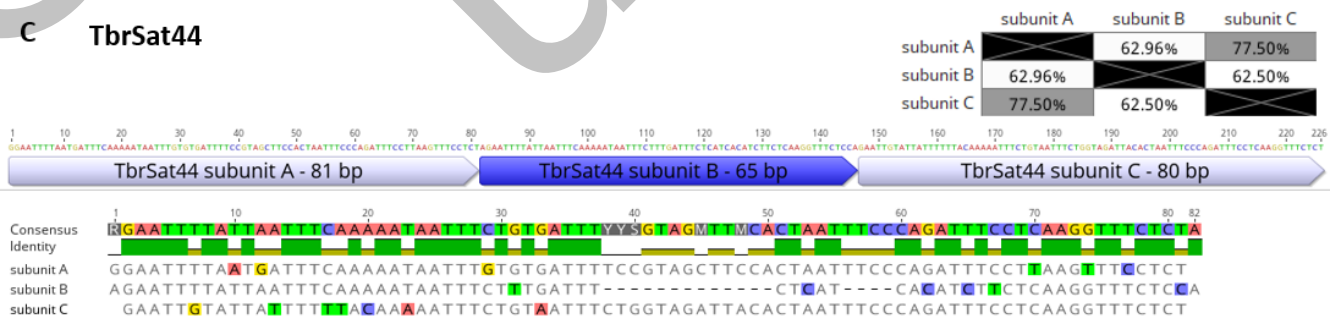
### A TbrSat09 – pairwise identity = 79.8%

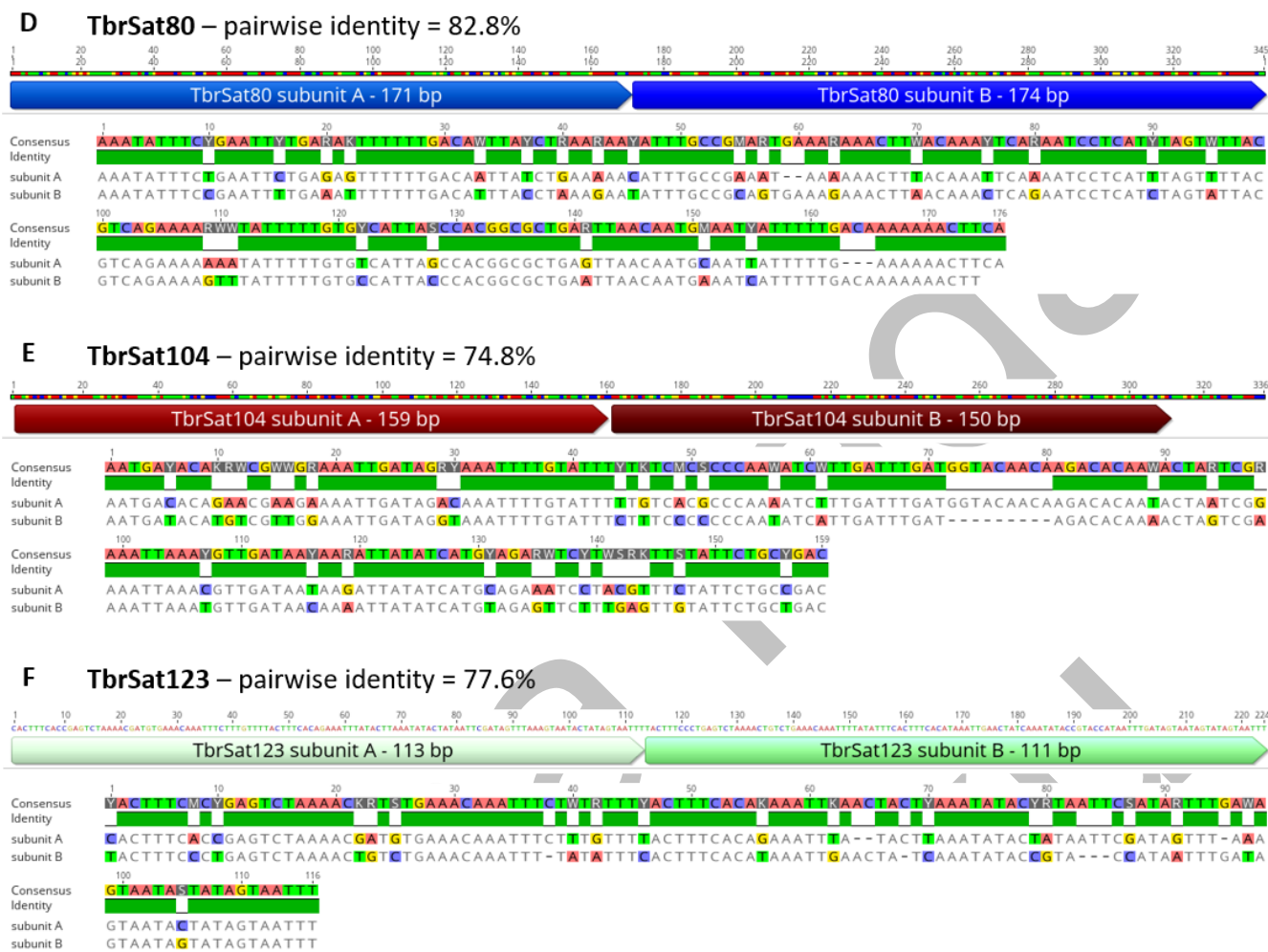


### B TbrSat16 – all subunits 13 bp



### C TbrSat44

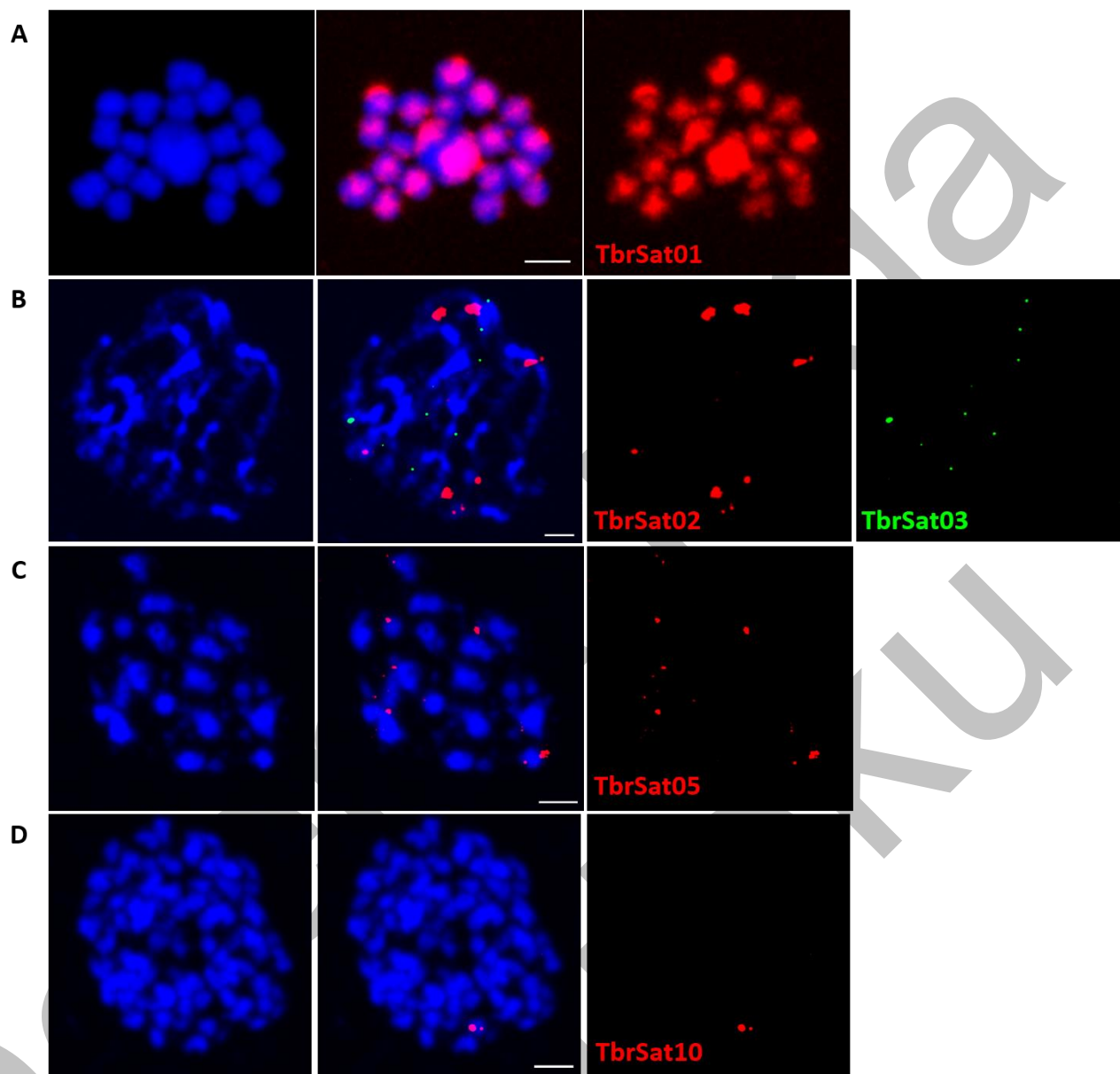




**Figure 31.** Schematic representation of the higher-order repeat structure of satellite DNAs TbrSat09 (A), TbrSat16 (B), TbrSat44 (C), TbrSat80 (D), TbrSat104 (E), and TbrSat123 (F). For each satellite DNA, Geneious alignments of the subunits are shown with subunits' pairwise identities indicated.

### 3.4.3. Chromosome localization of the *T. brevicornis* satellite DNAs

FISH experiments were performed to determine the chromosomal localization of *T. brevicornis* satDNAs. Major satDNA TbrSat01 is present on all of the chromosomes of the complement in large heterochromatic blocks (2n=18) (Figure 32A). Double FISH was performed with the TbrSat02 and TbrSat03 (superfamily A) specific probes to determine their location in regards to each other. The signals corresponding to TbrSat02 were scattered throughout the complement, with half of them being of higher intensity (Figure 32B). TbrSat03 were also scattered and showed a lower intensity than those of TbrSat02 overall, consistent with its five times lower genomic abundance. The signals did not colocalize (Figure 32B). TbrSat05 showed dispersed distribution throughout the genome (Figure 32C). In contrast, TbrSat10 was localized only in one place in the genome, possibly being chromosome specific (Figure 32D). The results are in accordance with their perspective genome abundance.



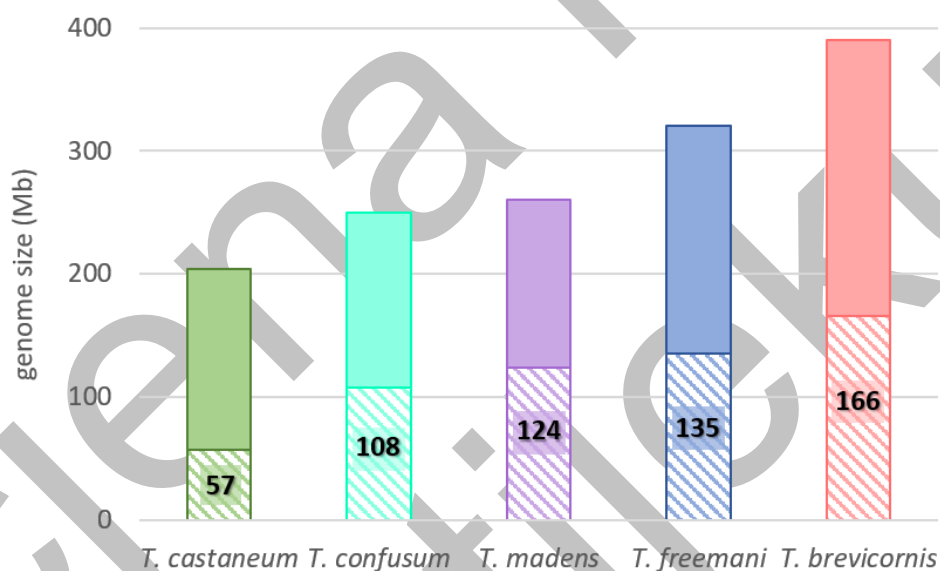
**Figure 32.** *Tribolium brevicornis* male chromosome spreads stained by DAPI (blue) and hybridized with FITC- (green fluorescence) and cy3-labelled (red fluorescence) probes specific for the satellite DNAs TbrSat01 (A), TbrSat02 and TbrSat03 (B), TbrSat05 (C) and TbrSat10 (D). Scale bar represents 3  $\mu$ m.



### 3.5. Comparative analyses of the *Tribolium* satellitomes

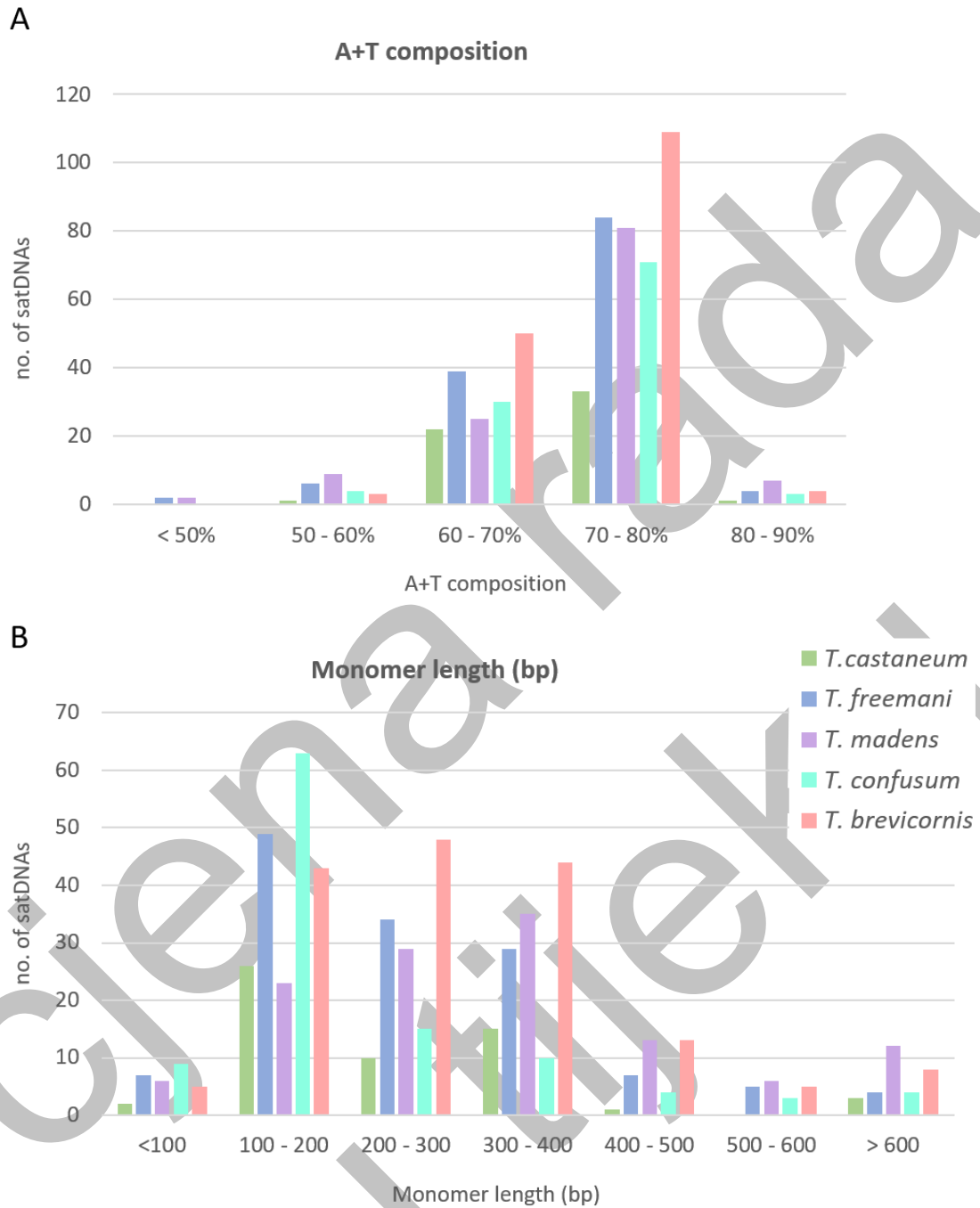
By analyzing the genomes of the four *Tribolium* species (*T. freemani*, *T. madens*, *T. confusum* and *T. brevicornis*) 533 satDNAs were identified in this study, with 528 of them being newly characterized. Together with the already characterized satellitome of *T. castaneum* which contains 57 identified satDNAs (Gržan et. al 2023), the total number of characterized satDNAs from the genus *Tribolium* is 590.

The relationship between the genome size of the five *Tribolium* species and the number of satDNAs identified in their genomes was investigated (Figure 33). Interestingly, the analysis showed that the species with the smallest genome of 204 Mb, *T. castaneum* (Figure 5), has the fewest characterized satDNAs (57) (Figure 33), while *T. brevicornis* has the largest genome of 390 Mb (Figure 5) and the highest number of identified satDNAs (166) (Figure 33). The remaining species also follow the same trend, with the number of satDNAs increasing with the size of the genome (Figure 33).



**Figure 33.** Comparison of the genome sizes of the five *Tribolium* species, portions of their satellitomes and the number of identified satellite DNAs. The striped bars represent the genome portion comprising the satellitome with the specific number of satellite DNAs in a genome being indicated.

Although the satellitomes of *Tribolium* species vary in number and genome proportion, the general characteristics of the satDNAs are similar. The majority of the characterized satDNAs have a high A+T composition, with 70-80% being the preferred proportion in all of the species (Figure 34A). Furthermore, the dominant length of the satDNAs ranges from 100 to 400 bp, with median length for the different species varying from 176 bp for *T. confusum*, 252 bp for *T. freemani*, 282 bp for *T. brevicornis* to 303 bp for *T. madens* (Figure 34B).



**Figure 34.** A+T composition (A) and monomer length (B) distribution of all of the characterized satDNAs in the genomes of *Tribolium freemani*, *Tribolium madens*, *Tribolium confusum* and *Tribolium brevicornis*.

Further comparative analyses of the newly defined satellitomes of *T. freemani*, *T. madens*, *T. confusum* and *T. brevicornis*, as well as the already known satellitome of *T. castaneum*, were aimed at finding possible similarities in their nucleotide sequences. The satDNAs that shared at least 60% similarity in their consensus sequences were determined to be orthologous (Table 15). Using the

above approach, 55 total orthologous satDNAs have been detected which were grouped into the 23 orthologous *Tribolium* satDNA families, named TribSAT1-23. Out of 23 orthologous TribSAT families, only the TribSAT1 family is shared by all five analyzed species, while the majority of orthologous families are mostly conserved in the pair of sibling species *T. castaneum* and *T. freemani*, and in the *castaneum* group in general (Table 15).

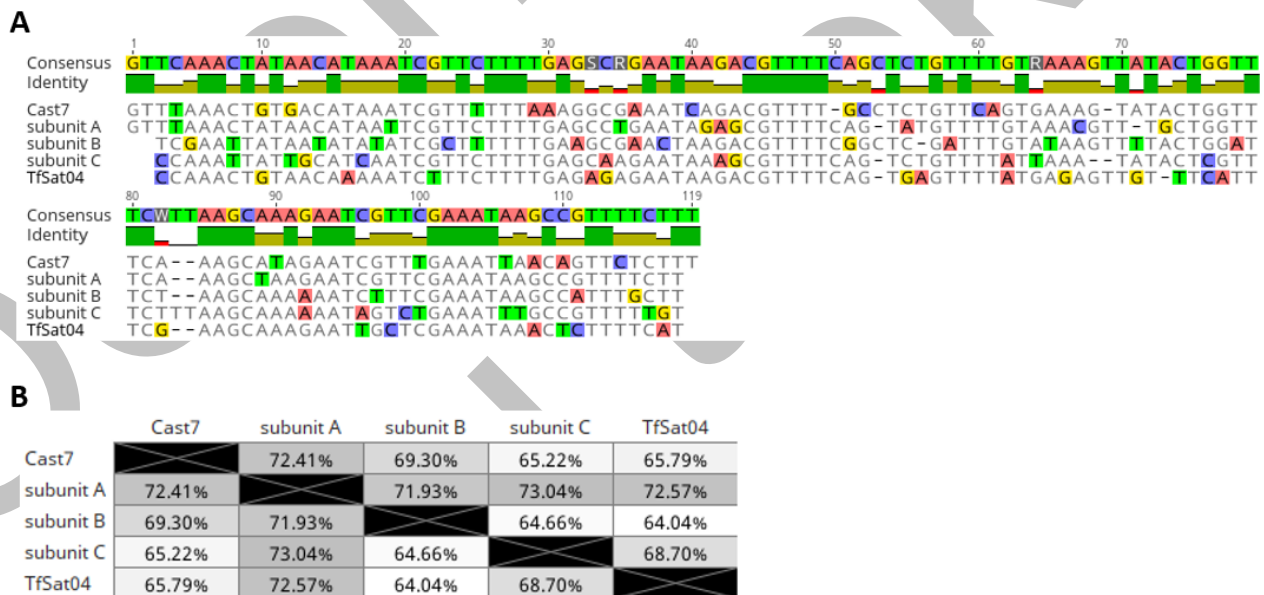
**Table 15.** List of the 23 orthologous satellite DNA families present in the five species of the genus *Tribolium*. The color-coded columns list the names of the satellite DNAs as they were identified and named in the satellitomes of the individual species. Diagonal stripes indicate the partially similar satellite DNA sequences whose consensus sequences differ in length.

orthologous satDNA family	castaneum group			confusum group	brevicornis group
	<i>T. castaneum</i>	<i>T. freemani</i>	<i>T. madens</i>	<i>T. confusum</i>	<i>T. brevicornis</i>
TribSAT1	TCsat17	TfrSat08	TmaSat04	TcoSat09	TbrSat23
TribSAT2	TCsat30	TfrSat76	TmaSat14	TcoSat45	
TribSAT3	TCsat15	TfrSat02	TmaSat03		
TribSAT4	TCsat32	TfrSat111		TcoSat37	
TribSAT5	Cast7	TfrSat03/04			
TribSAT6	Cast4	TfrSat05			
TribSAT7	TCsat23	TfrSat15			
TribSAT8	TCsat12	TfrSat25			
TribSAT9	Cast6/TCsat36	TfrSat27			
TribSAT10	Cast8	TfrSat07			
TribSAT11	Cast9	TfrSat22			
TribSAT12	TCsat13	TfrSat11			
TribSAT13	TCsat29	TfrSat42			
TribSAT14	Cast1	TfrSat102			
TribSAT15	TCsat19	TfrSat101			
TribSAT16		TfrSat10	TmaSat05		
TribSAT17		TfrSat13	TmaSat13		
TribSAT18		TfrSat67	TmaSat09		
TribSAT19		TfrSat78	TmaSat87		
TribSAT20		TfrSat121	TmaSat53		
TribSAT21		TfrSat30		TcoSat48	
TribSAT22		TfrSat06			TbrSat56
TribSAT23			TmaSat16		TbrSat20

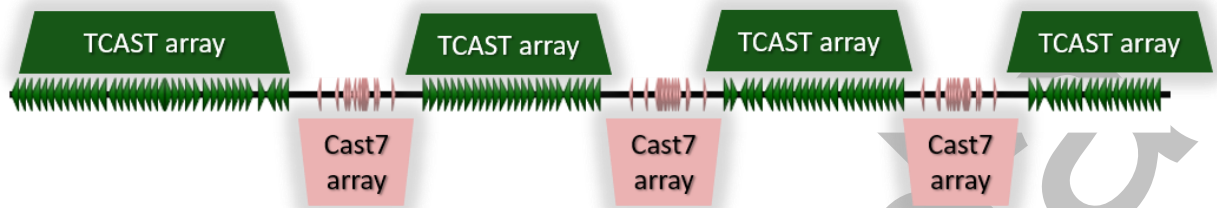
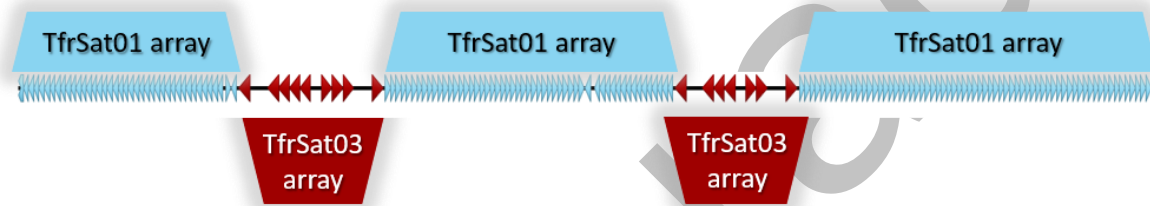
### 3.5.1. Orthologous satDNAs of the sibling species *T. castaneum* and *T. freemani*

The sibling species *T. castaneum* and *T. freemani* share 15 orthologous satDNA families with 11 families that occur only between them (Table 15). A detailed analysis of the most prominent orthologs was performed to gain insight into the evolution of satDNAs between these closely related species.

First of all, the orthologous TribSAT5 family proved to be particularly interesting. Namely, satDNAs TfrSat03/04 from the *T. freemani* superfamily A have an orthologous sequence in the *T. castaneum* genome, which is known as satDNA Cast7 (Table 15). The alignment of the consensus sequences Cast7, TfrSat03 subunits A/B/C and TfrSat04 showed pairwise identities from 64% to 73% (Figure 35A and 35B), with no apparent grouping of sequences by similarity. When the long-range organization of the satDNAs was examined, Cast7 was found to have a similar organizational pattern to TfrSat03 (Figure 35C). It is present in the short arrays, with the monomers changing orientation, and is embedded in larger arrays of TCAST, the major satDNA of *T. castaneum* (Volarić et al. 2024). Regardless of the similar organization pattern between TCAST-Cast7 and TfrSat01-TfrSat03 arrays (Figure 35C), it should be noted that the major satDNA TCAST of *T. castaneum* has no nucleotide sequence similarity with the major satDNA TfrSat01 of *T. freemani*.

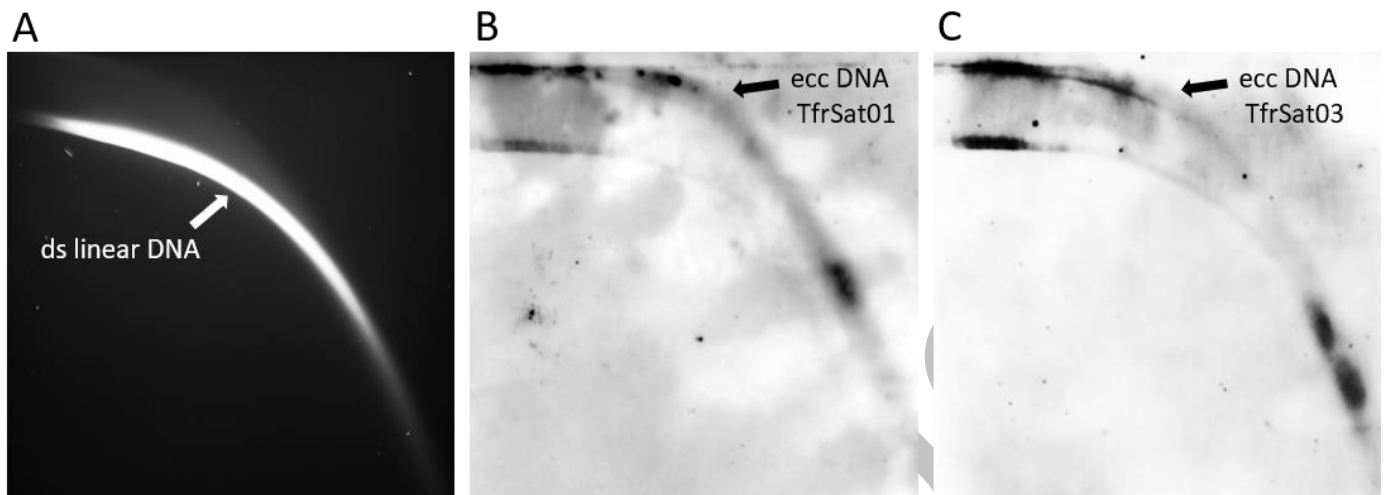


C

*T. castaneum**T. freemani*

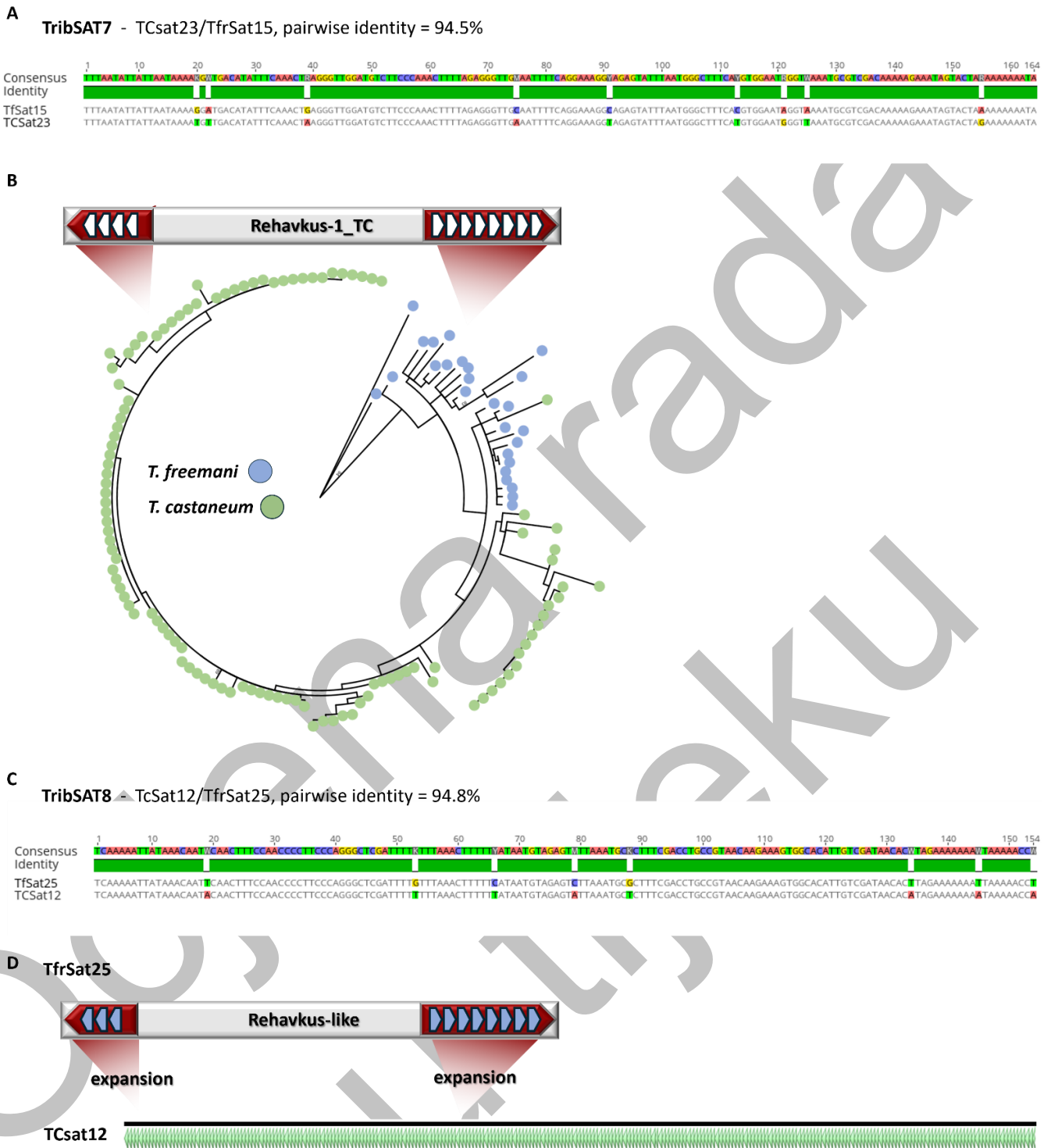
**Figure 35.** Geneious alignment (A) and distance matrix (B) of the consensus sequences of Cast7, TfrSat03 subunits A, B and C and TfrSat04. Graphic view of the long-range organization of TCAST-Cast7 arrays in the *Tribolium castaneum* genome assembly and TfrSat01-TfrSat03 arrays in the *Tribolium freemani* genome assembly (C).

Since it has been shown *in situ* that TfrSat01-TfrSat03 arrays form the (peri)centromeres of all chromosomes except the male sex chromosome  $y_p$ , the question arose as to what mechanism could be responsible for their spread between non-homologous chromosomes. One of the ways of such spreading could be via eccDNAs originated by amplification and recombination-based excision from satellite repeat arrays. To explore possible mechanisms of propagation of TfrSat03 in the context of larger arrays of TfrSat01, 2D agarose gel electrophoresis followed by Southern blot hybridization was performed to detect eccDNAs containing satDNAs TfrSat01 and TfrSat03 (Figure 36). Indeed, both satDNAs were found to be present in eccDNAs, suggesting that TfrSat01/TfrSat03-containing eccDNAs may contribute to the expansion and homogenization of these satellites through their reinsertion into chromosomal DNA (Figure 36).



**Figure 36.** Two-dimensional gel electrophoresis of *Tribolium freemani* genomic DNA stained by ethidium bromide (A) and followed by Southern blot hybridization with probes specific for satellite DNAs TfrSat01 (B) and TfrSat03 (C). The white arrow in panel A indicates the signal originating from chromosomal double-stranded DNA (ds linear DNA), while the black arrows in panels B and C indicate the signals originating from extrachromosomal circular DNA molecules containing TfrSat01 and TfrSat03 satDNA sequences.

Further investigation of the orthologous satDNAs between the siblings showed that the satDNAs of TribSAT7 and TribSAT8 are related to the transposon family called Rehavkus (Suppl. table 2). The Rehavkus superfamily, initially identified in *T. castaneum* (Kapitonov et al. 2006) consists of long terminal inverted repeats with subterminal tandem repeats. TCsat23 has already been described in the genome of *T. castaneum* as a repeat unit present in the inverted repeats of the Rehavkus-1\_TC DNA transposons (Gržan et al. 2023). TCsat23 and TfrSat15 are 94.5% similar in their consensus sequence (Figure 37A), and like TCsat23, TfrSat15 is also present in the inverted termini in the Rehavkus-1\_TC-like DNA transposon in the *T. freemani* genome (Figure 37B). The monomers of TfrSat15 and TCsat23 were extracted from the inverted termini of their respective Rehavkus-1\_TC elements, and a phylogenetic maximum likelihood analysis was performed. Monomers extracted from the same genome clustered together, suggesting that the satDNAs of TribSAT7 evolve according to the concept of concerted evolution (Figure 37B). As for TribSAT8, TfrSat25 is also found as a tandem repeat in the inverted termini of Rehavkus-like DNA element with 2 - 12 tandemly repeated copies (Figure 37D). Remarkably, its ortholog in *T. castaneum*, TCsat12, is organized in the form of long satDNA arrays stretching up to 80 kb (Figure 37D). A possible explanation lies in the intensive propagation of TCsat12 in *T. castaneum* that occurred after speciation of the sibling species. However, the pairwise similarity of the TribSAT8 satDNA pair TfrSat25-TCsat12 remains remarkably high 94.5% (Figure 37C).

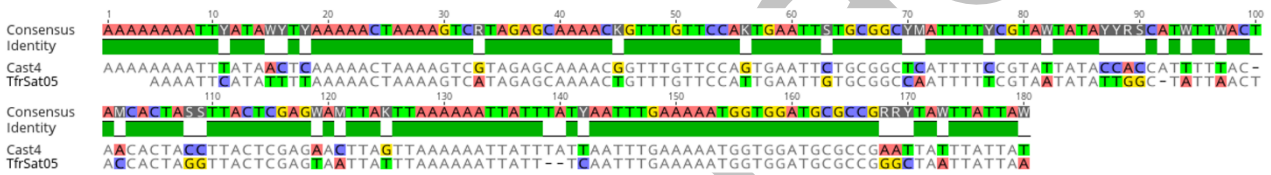


**Figure 37.** Relationship of the orthologous families TribSAT7 and TribSAT8 with Rehavkus DNA transposons. A) Geneious alignment of the satellite DNAs from TribSAT7 family, TfrSat15 and TCsat23. B) Schematic view of Rehavkus -1\_TC DNA element with its inverted termini containing satDNAs of TribSAT7 family, and the Maximum Likelihood tree of 138 TribSAT7 monomers extracted from the *Tribolium castaneum* and *Tribolium fremani* genomes with the bootstrap value of 1000. The repeats originating from the two genomes are color-coded and shown with blue (*T. fremani*) and green (*T. castaneum*) dots. C) Geneious alignment of the satDNAs from TribSAT8 family, TfrSat25 and TCsat12. D) Schematic view of the Rehavkus-like element containing TfrSat25 in *T. fremani*, and its orthologue TCsat12 that underwent intense propagation into 80 kb long array in the *T. castaneum* genome.



The pairwise identity of the remaining orthologous satDNA families between *T. castaneum* and *T. freemani* (TribSAT6/9/10/11/12/13/14/15) is between 63.8% and 82.2% (Figure 38). The monomers of the satDNAs of the TriSAT14 and TriSAT15 families differ in length, but have common conserved sequence regions (Figure 38G and 38H). In TribSAT14, Cast1 is 172 bp long, whereas TfrSat102 is 326 bp long, harboring the conserved Cast1 (Figure 38G). The same pattern is observed in the orthologous family TribSAT15, containing 618 bp long TCsat19, and 151 bp long TfrSat101, which overlaps with 66.9% similarity with part of the TCsat19 sequence (Figure 38H).

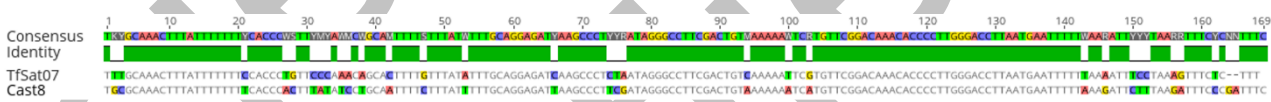
**A TribSAT6 - Cast4/TfrSat05, pairwise identity = 80.7%**



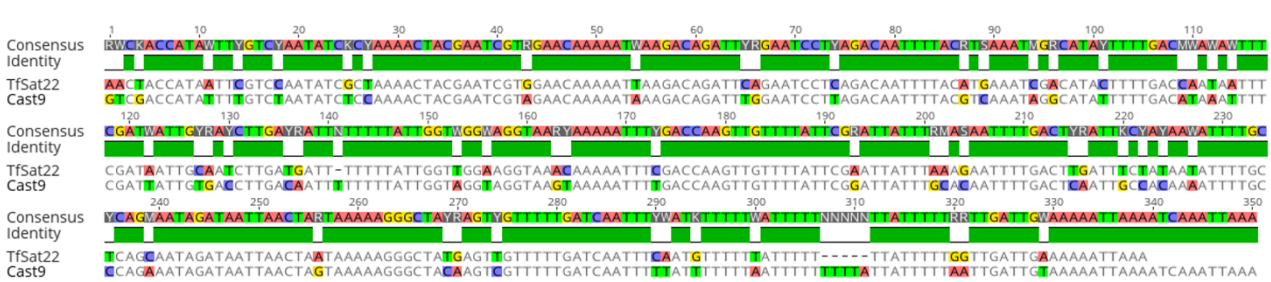
**B TribSAT9 - Cast6/ TCsat36/ TfrSat27**



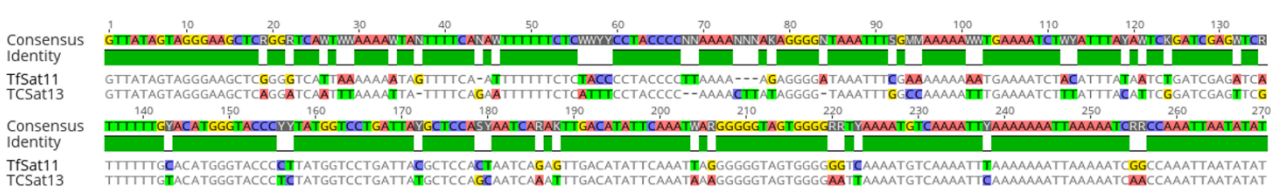
**C TribSAT10 - Cast8/TfrSat07, pairwise identity = 81.5%**



**D TribSAT11 - Cast9/TfrSat22, pairwise identity = 81.7%**

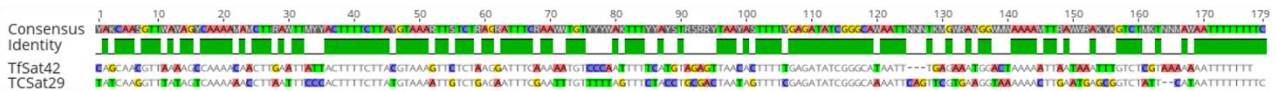


**E TribSAT12 - TCsat13/TfrSat11, pairwise identity = 82.2%**

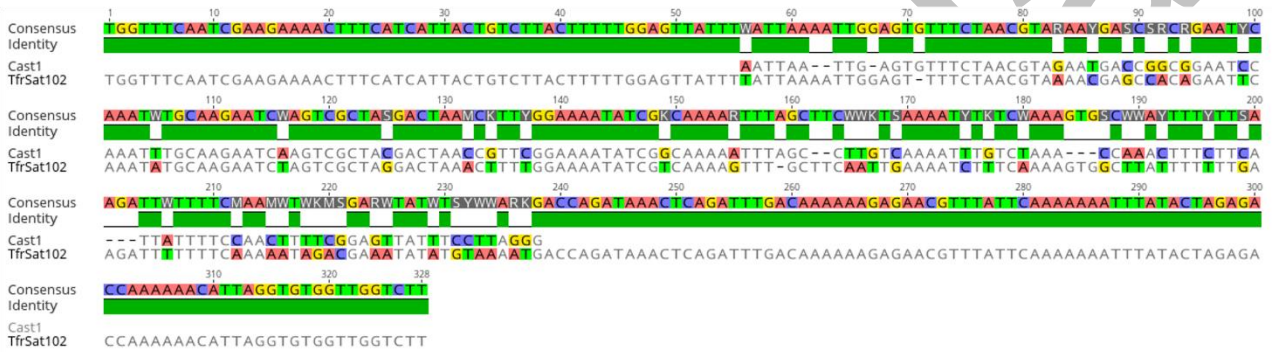




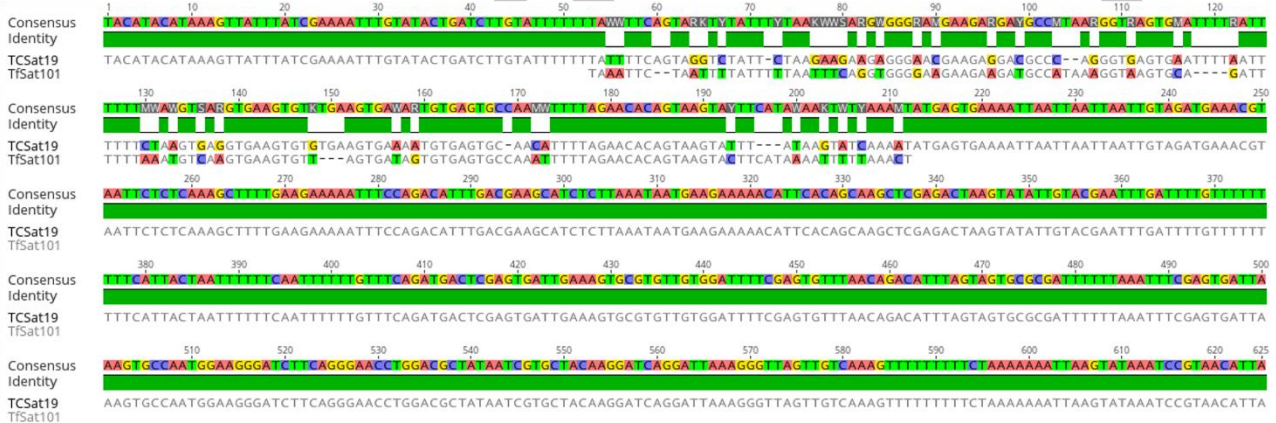
**F TribSAT13 - TcSat29/TfrSat42, pairwise identity = 63.8%**



**G TribSAT14 - Cast1/TfrSat102, pairwise identity = 67.8%**



**H TribSAT15 - TcSat19/TfrSat101, pairwise identity = 66.9%**

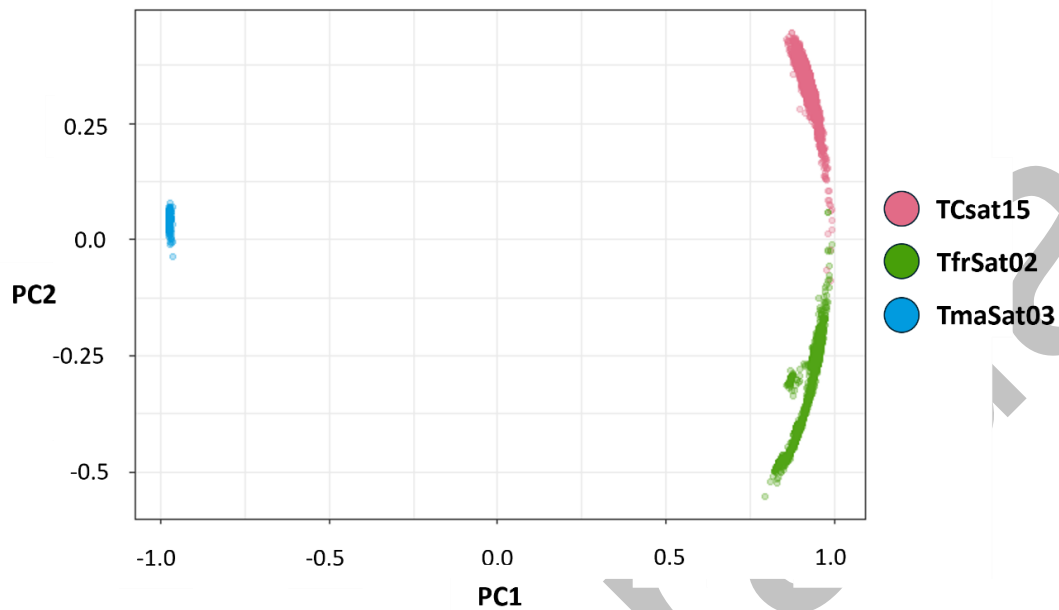


**Figure 38.** Geneious alignments of the *Tribolium castaneum* / *Tribolium freemani* orthologous satellite DNA families TribSAT6 (A), TribSAT9 (B), TribSAT10 (C), TribSAT11 (D), TribSAT12 (E), TribSAT13 (F), TribSAT14 (G) and TribSAT15 (H).

**3.5.2. Orthologous satDNAs of the species of *castaneum* group**

From the pool of the *Tribolium* orthologous satDNA families, only one family, called TribSAT3, is common to all studied species of the *castaneum* group, namely *T. castaneum*, *T. freemani* and *T. madens* (Table 15). TribSAT3 is composed of the satDNAs TcSat15, TfrSat03 and TmaSat02, whose consensus similarities range from 66.5% to 85.7% (Figure 39). Principal component analysis (PCA) was performed for all monomers of TribSAT3 extracted from the genome assemblies with a similarity criterion of 70% (Figure 40). The monomers extracted from the genomes of *T. castaneum* and *T.*

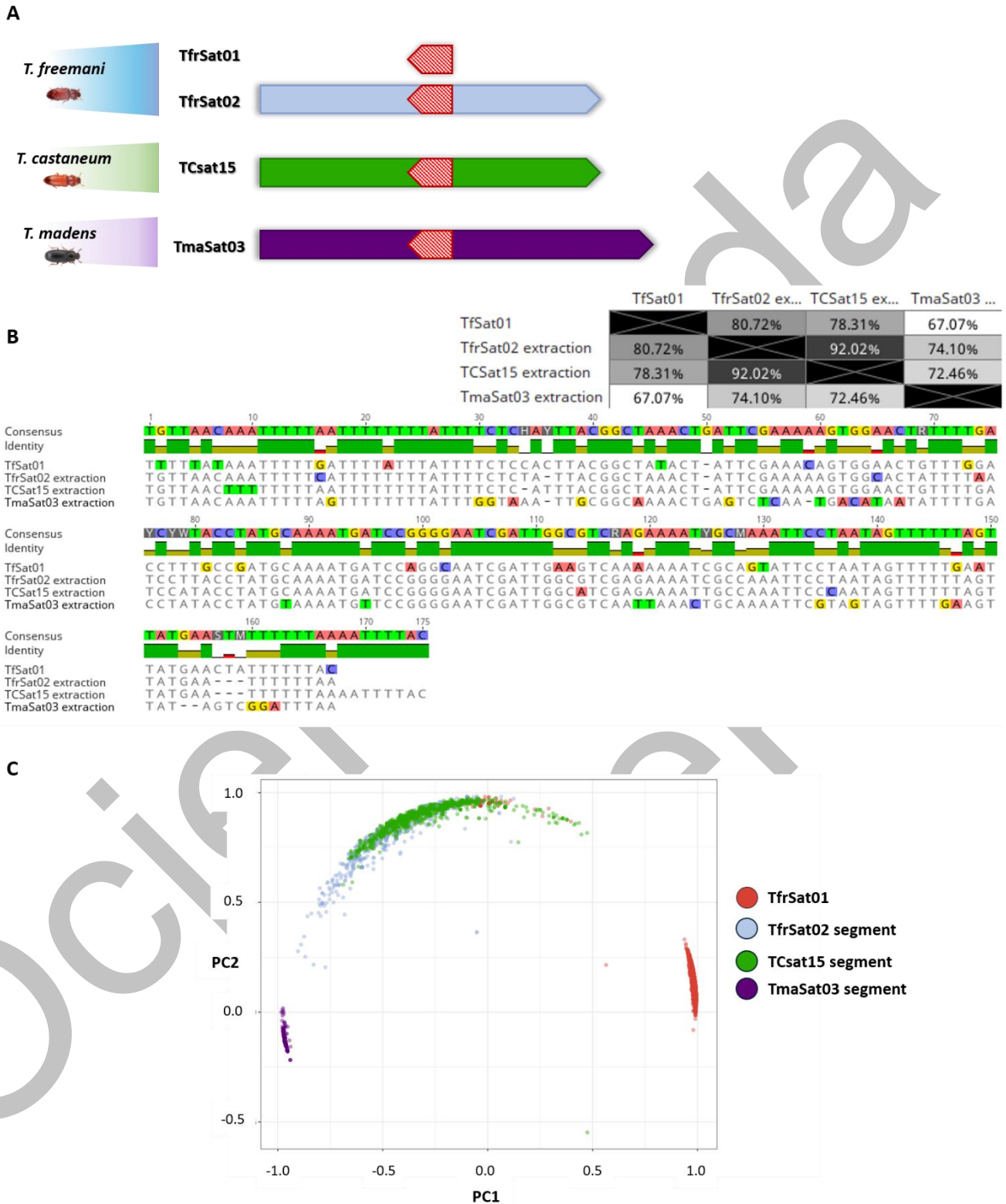




**Figure 40.** The principal component analysis of the monomers of orthologous satellite DNAs TCsat15, TfrSat02 and TmaSat03, extracted from the genomes of *Tribolium castaneum*, *Tribolium freemani*, and *Tribolium madens*, respectively. The dots represent monomers extracted from the three species according to the color-coded legend.

Interestingly, the TribSAT3 orthologous family is based on satDNAs, which represent the satellites with the longest repeat units in all three species. In addition to their length, monomers of these satDNAs are characterized by another interesting feature. The detailed investigation of the TCsat15, TfrSat02, and TmaSat03 monomer sequences revealed that each of these monomers is comprised of a 166 bp long segment, that corresponds to the *T. freemani* major satDNA TfrSat01 monomer (Figure 41A). Based on the consensus' alignment, TfrSat01 nucleotide sequence shows the greatest similarity with the segment extracted from TfrSat02 (80.7%). The segment extracted from TCsat15 is slightly more divergent (78.3%), while the segment extracted from TmaSat03 is the most divergent (67.1%) (Figure 41B). The PCA analysis of the randomly subsampled monomers of TfrSat01 and corresponding segments from TfrSat02, TCsat15 and TmaSat03 was performed. Monomers of TfrSat01 grouped together, as well as the monomers extracted from TmaSat03, representing the opposite ends of the PCA dimensions. Segments extracted from TfrSat02 and TCsat15 showed distinct grouping but remained closer together with a few interspersed TfrSat01 monomers (Figure 41C). Considering that the results follow phylogenetic relationships between the species and that TfrSat01 does not show tandemization in any species other than *T. freemani*, it can be concluded that TfrSat01 as a satDNA is a unique trait of the *T. freemani* genome and is not found in the *Tribolium* common ancestor.

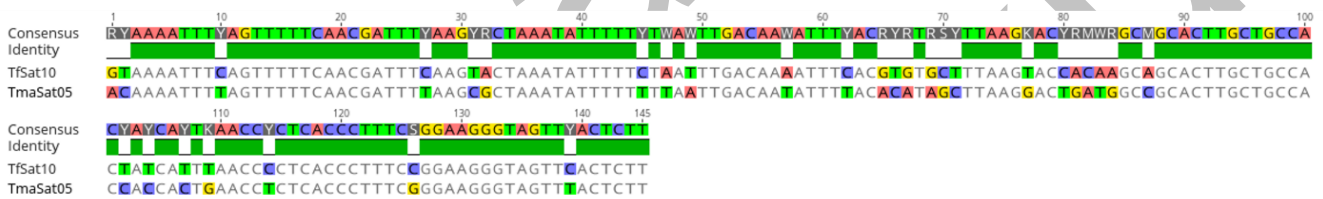




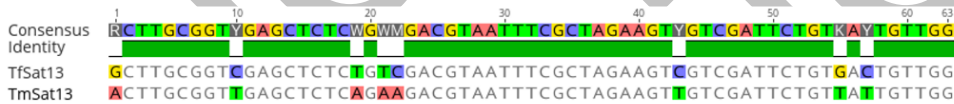
**Figure 41.** A) Schematic view of TribSAT3 satellite DNA TfrSat02, TCsat15 and TmaSat03 monomers, with embedded segment corresponding to the *Tribolium freemani* major satDNA TfrSat01 (marked by red arrow pentagon). B) Geneious alignment and distance matrix of TfrSat01 and corresponding segments from the three TribSAT3 satDNAs TfrSat02, TCsat15, and TmaSat03. C) The principal component analysis of monomers of TfrSat01 and corresponding segments from the three TribSAT3 satDNAs TfrSat02, TCsat15, and TmaSat03. The dots represent monomers extracted from the three species according to the color-coded legend.

Five different orthologous families are shared by the species *T. fremani* and *T. madens* – TribSAT16/17/18/19/20 (Table 15). The pairwise identity of satDNAs within the family ranges from 63.2% to 87.3% (Figure 42). Thereat, the orthologous families with the biggest similarities are TribSAT16, TribSAT17 and TribSAT18 (78.6% - 87.3%) in which satDNAs share comparable monomer lengths (Figure 42A – 42C). In contrast, orthologous satDNAs classified into TribSAT19, namely TfrSat78 (283 bp) and TmaSat87 (276 bp), show fewer similarities despite similar monomer lengths (63.2%) due to the differential distribution of similarity along the monomer sequence (Figure 42D). The TribSAT20 satDNAs differ in length, with TfrSat121 being 426 bp long and TmaSat53 being 297 bp long and corresponding to the central part of the TfrSat121 consensus (Figure 20E). There is a possibility that the aforementioned orthologous families have their counterparts in the genome of *T. castaneum*, but their genomic abundance was too low to detect them.

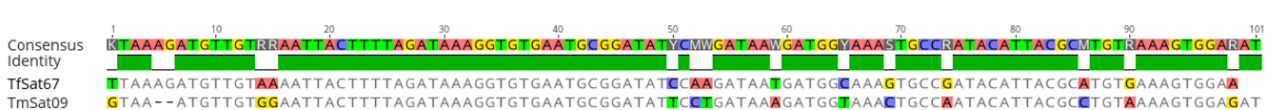
**A TribSAT16 - TfrSat10/TmaSat05, pairwise identity = 78.6%**



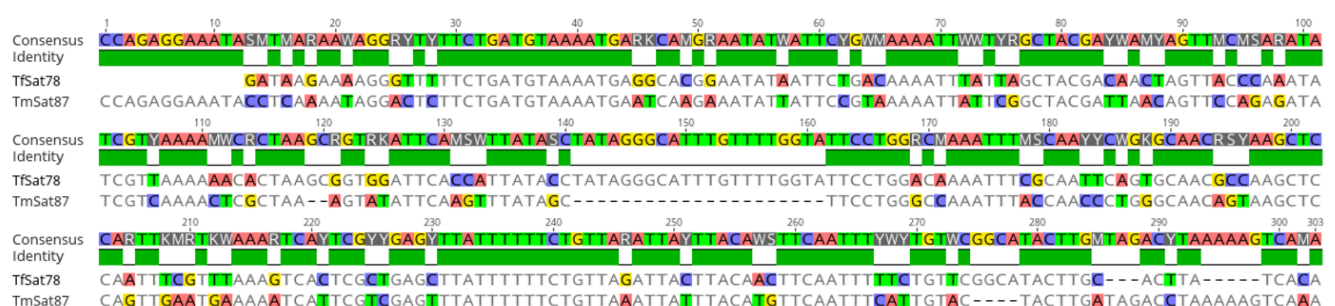
**B TribSAT17 - TfrSat13/TmaSat13, pairwise identity = 87.3%**



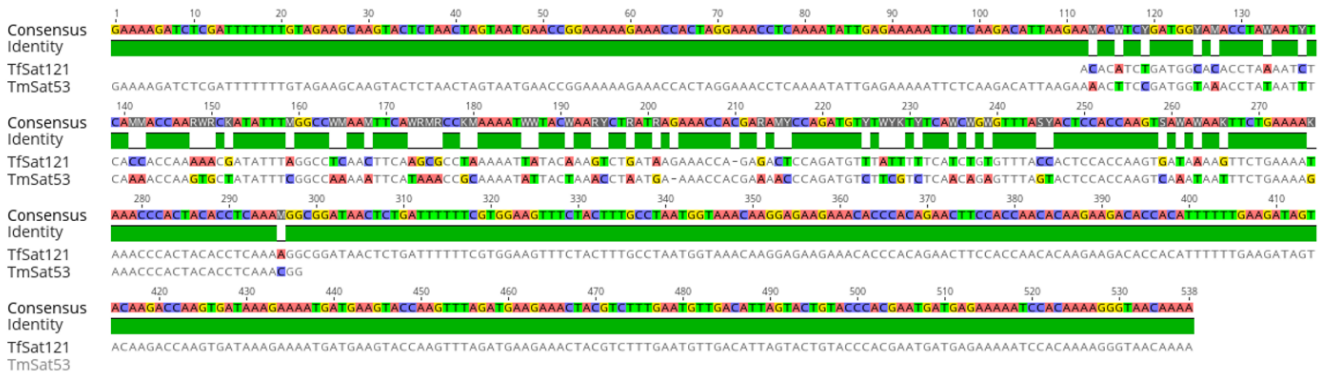
**C TribSAT18 - TfrSat67/TmaSat09, pairwise identity = 84.8%**



**D TribSAT19 - TfrSat78/TmaSat87, pairwise identity = 63.2%**



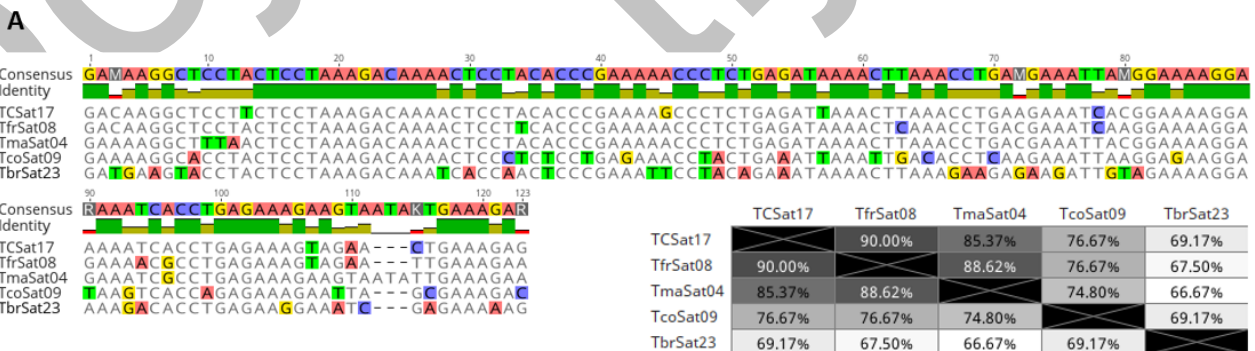
**E TribSAT20 - TfrSat121/TmaSat53, pairwise identity = 72.7%**

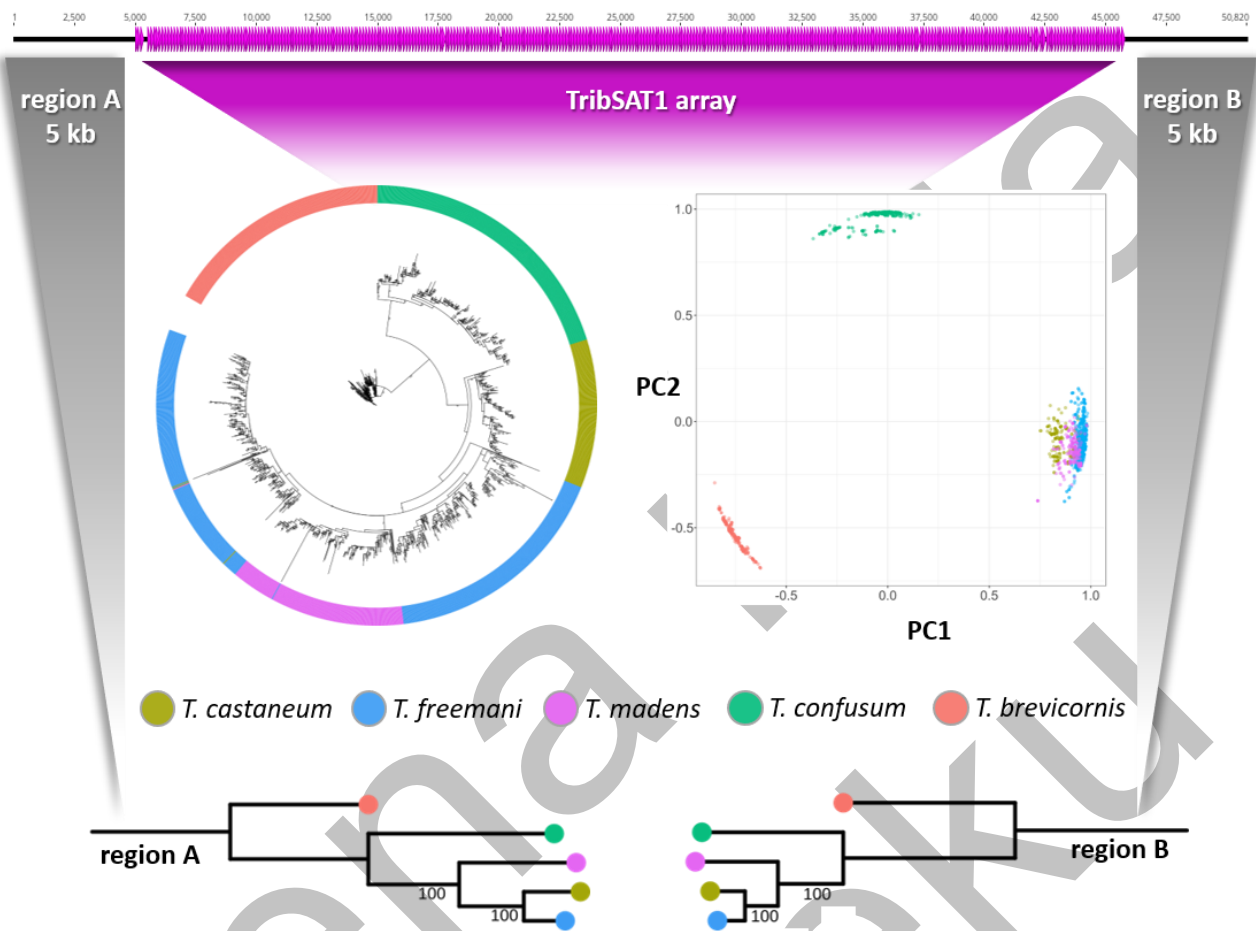


**Figure 42.** Geneious alignments of the orthologous satellite DNA from the families TribSAT16 (A), TribSAT17 (B), TribSAT18 (C), TribSAT19 (D) and TribSAT20 (E), shared by *Tribolium freemani* and *Tribolium madens*.

**3.5.3. Orthologous satDNAs of the species of *castaneum/confusum/brevicornis* group**

There is a satDNA found in all examined species of the genus *Tribolium*, making it a potential ancestral satDNA. This satDNA is referred to as the TribSAT1 family, comprising satDNAs TCsat17, TfrSat08, TmaSat04, TcoSat09 and TbrSat23 (Table 15). Overall, the species-specific consensus sequences are quite uniform in length and range from 120 bp to 123 bp (Figure 43A). Regarding the nucleotide sequence, the similarities between the satDNAs range from 66.7% to 90.0% (Figure 43A).



**B**

**Figure 43.** Relationships between orthologous satellite DNA from the family TribSAT1 shared by five *Tribolium* species, *Tribolium castaneum*, *Tribolium freemani*, *Tribolium madens*, *Tribolium confusum* and *Tribolium brevicornis*. A) Geneious alignment and distance matrix of the satellite DNAs TCsat17, TfrSat08, TmaSat04, TcoSat09, and TbrSat23. B) Schematic representation of the genomic organization of TribSAT1 and 5 kb flanking regions. Maximum likelihood tree and principal component analysis of 2025 TribSAT1 monomers extracted from the *Tribolium* genomes are shown in the center panels. The maximum likelihood trees of the 5 kb flanking regions (region A on the left, region B on the right) are shown in the bottom panel. The color-coded legend indicates the species to which the analyzed sequences belong.

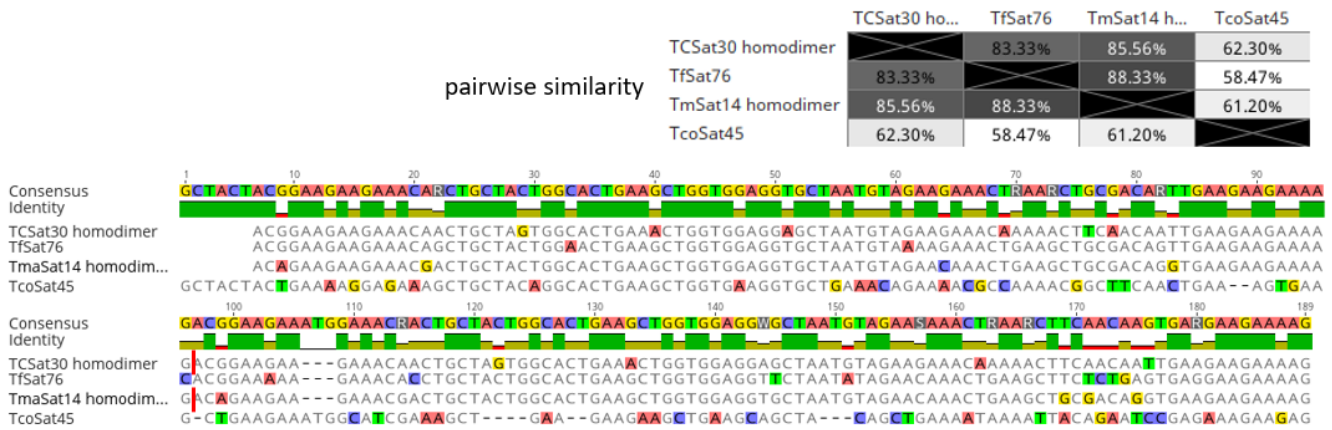
A total of 2025 monomers of TribSAT1 satDNAs were extracted from the genome assemblies of the respective species. Of these, 290 monomers belong to *T. castaneum*, 661 to *T. freemani*, 253 to *T. madens*, 465 to *T. confusum* and 356 to *T. brevicornis*. Two approaches were used to analyze the mutual relationships of congeneric monomers: maximum likelihood analysis, and principal component analysis. The results of the two analyses were consistent, and showed that monomers extracted from the same species grouped together (Figure 43B). Furthermore, the grouping of monomers was consistent with the phylogenetic relationships between *Tribolium* species, revealing that the

monomers extracted from the *castaneum* group cluster together (Figure 43B). Regarding the two remaining species, maximum likelihood analysis showed that the monomers extracted from *T. confusum* are somewhat more closely related to monomers of the *castaneum* group than those of *T. brevicornis*. These results suggest that the TribSAT1 family evolves according to the concept of concerted evolution. Interestingly, TribSAT1 is organized in a long array spanning up to 40 kb in all species studied (Figure 43B). This was also confirmed in previous FISH experiments with TribSAT1 satDNAs, which only showed a signal on one chromosome of the complement (Figure 19B, 27D). The annotation of the genome assemblies of *T. castaneum* Tcas5.2 and *T. freemani* Tfree1.0 indicates that the chromosome in question is chromosome 3, so it can be speculated that TribSAT1 is a conserved marker for chromosome 3 of the genus *Tribolium*. Further analysis was performed on the flanking regions of the TribSAT1 arrays, extracting 5 kb upstream and downstream of the satDNAs. The sequences confirmed syntenic loci, so further maximum likelihood analyses of the flanking regions were performed. As with the satDNA monomers, the phylogenetic trees of left and right flanking regions is in accordance with the evolutionary relationships between species (Figure 43B). To be more precise, the relationship between the species of the *castaneum* group were supported with 100% bootstrap value, while the flanking regions of *T. brevicornis* were the most divergent.

TribSAT2 is a satDNA family shared by four species of the genus – *T. castaneum*, *T. freemani*, *T. madens* and *T. confusum* (Table 15). A notable difference between the satDNAs of each species is the length of the consensus monomer. While the monomers of TCsat30 and TmaSat45 are both 90 bp long (Gržan et al. 2023) (Table 10), TfrSat76 is 190 bp long (Table 8) and TcoSat45 is 177 bp long (Table 12). It has already been mentioned that the monomer of TfrSat76 has a complex organization with two subunits that are similar to each other, making it a dimeric HOR (Figure 12C). Therefore, the monomers of TCsat30 and TmaSat45 were artificially made into homodimers to obtain a more accurate alignment score (Figure 44). The pairwise similarities of the TribSAT2 satDNAs range from 58.5% to 88.3%, with the consensus of TcoSat45 being the most divergent, which is expected based on the phylogeny (Figure 44).



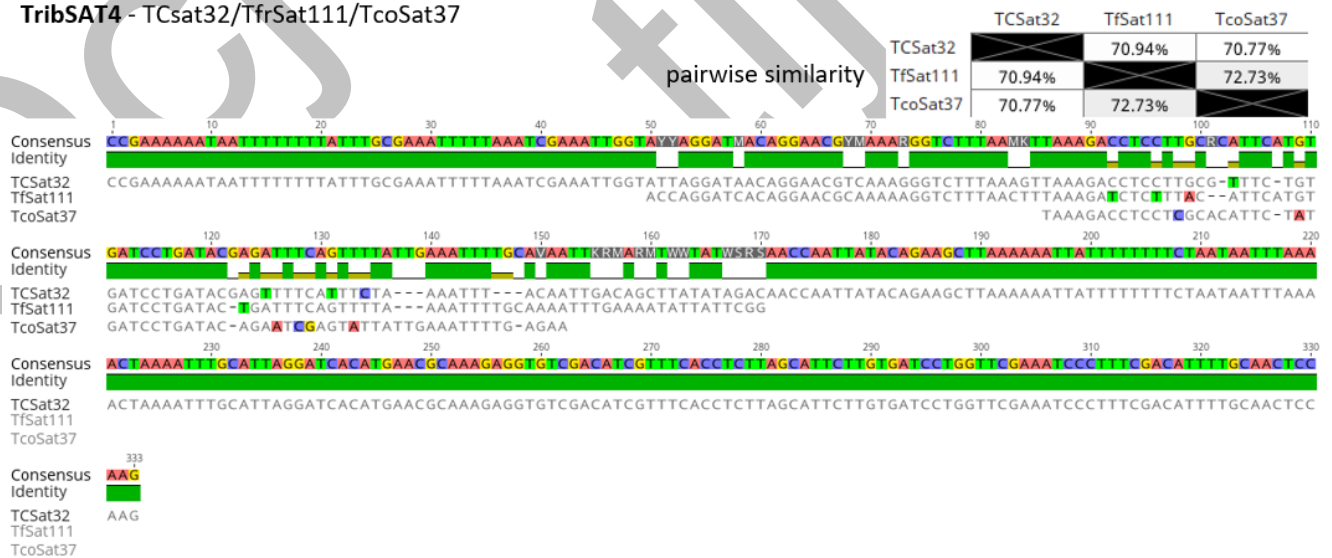
**TribSAT2 - TCsat30 homodimer/TfSat76/TmaSat14 homodimer/TcoSat45**



**Figure 44.** Geneious alignment and distance matrix of the orthologous satellite DNAs TCsat30, TfSat76, TmaSat14 and TcoSat45 from the family TribSAT2 shared by four *Tribolium* species (*Tribolium castaneum*, *Tribolium freemani*, *Tribolium madens*, and *Tribolium confusum*, respectively). The consensus sequences of the TCsat30 and TmaSat14 satDNAs are shown as dimers for easier comparison with other sequences, and the red lines represent the borders of monomers.

The orthologous satDNA family TribSat4 was found in the genome of three species, namely *T. castaneum*, *T. freemani* and *T. confusum* (Table 15). Remarkably, the satDNA monomer was not conserved in its entirety between species, with the monomer of TCsat32 being 325 bp long (Gržan et al. 2023), TfrSat111 is 115 bp long (Table 8) and TcoSat37 is 64 bp long (Table 12). The pairwise similarities in the corresponding parts of the satDNA consensuses are rather similar, and range from 70.8% to 72.7% (Figure 45).

**TribSAT4 - TCsat32/TfrSat111/TcoSat37**



**Figure 45.** Geneious alignment and distance matrix of the orthologous satellite DNA family TribSAT4 shared by the three *Tribolium* species, *Tribolium castaneum*, *Tribolium freemani* and *Tribolium confusum*.



In general, the analyses of orthologous satDNAs from the *Tribolium* species have shown that the similarities of orthologous sequences in most cases reflect phylogenetic relationships within the genus. Some orthologous satDNAs have shown that, in addition to the accumulation of nucleotide changes, their sequences can also exhibit considerable differences in the length and complexity of the repeat units.

Ocjena rada  
u tijeku

## 4. DISCUSSION

Ocjena rada  
u tisku

The aim of this study was to comprehensively analyze the satellitomes of the four insect species of the genus *Tribolium*. Due to the well-defined evolutionary relationships between them and a high proportion of satDNAs in their genomes, the species of the genus *Tribolium* are one of the best models for the study of the structure, organization and evolution of satellitomes.

### **The composition of *Tribolium* satellitomes**

The initial hypothesis of this research was that, besides the major, dominant satDNAs, the genomes of the *Tribolium* species are abundant in low-copy satDNAs. The major satDNAs of the eight species have been previously identified by classical molecular biology methods, such as restriction digestion, Southern blot, and molecular cloning of the few available satDNA monomers (Juan et al. 1993; Plohl et al. 1993; Ugarković et al. 1996a; Ugarković et al. 1996b; Mravinac et al. 2004; Mravinac et al. 2005; Mravinac and Plohl 2010). Due to the limited sensitivity of the methods used at the time, only the satDNAs with a high-copy number, which dominate the genomes of the species, could be detected and, in the case of *Tribolium*, account for up to 60% of the genome (Mravinac and Plohl 2010). However, despite the high genomic abundance of major satDNAs, the question remains whether the genomes of *Tribolium* beetles hide other tandemly repeated sequences.

The true renaissance of the satDNA field happened with the emergence of high-throughput genomic sequencing, starting with NGS and followed by highly accurate long-range sequencing by PacBio HiFi technology. The benefits of second and third generation sequencing were used in this study. Consequently, this study provided a comprehensive analysis of the satellitomes of the four *Tribolium* species, *T. freemani*, *T. madens*, *T. confusum* and *T. brevicornis*, by combining all the techniques noted above. Firstly, the *Tribolium* genomes were sequenced using Illumina and PacBio HiFi technology, and 533 satDNAs were described by combining experimental and bioinformatics methods, with satellitomes spanning from 17.54% to 41.38% of *Tribolium* genomic content. Graph-based clustering of short Illumina reads through a bioinformatics pipeline confirmed the major satDNAs as the dominant sequences in the four species studied. However, *in silico* analyses revealed that the genomic abundance of some of the major satDNAs is lower than experimentally determined by dot-blot hybridization. For example, TcoSat01 was experimentally found to account for 40% of the genome (Plohl et al. 1993), whereas the bioinformatic assessment was closer to 13%. Furthermore, the genomic abundance of TbrSat01 was experimentally estimated to be 21% (Mravinac et al. 2005), while *in silico* analysis showed a proportion of 14%. The dot-blot analyses performed in the original research to estimate satDNA abundance are used to determine the relative amount of target DNA, as they require well-defined amounts of control DNA for comparison. For this reason, the method is not acutely precise and may lead to overestimation. As the bioinformatic analyses used high-coverage WGS data obtained by two different sequencing technologies, and the results for the *T. freemani* and

*T. madens* major satDNAs were in accordance with experimental measurements, the *in silico* estimates of satDNA abundance seem more plausible.

The newly characterized satellitomes of four *Tribolium* species all contain more than 100 different satDNAs, between 108 and 166 in total. The satellite profiles of all of them follow the same trend: one dominant satDNA with high copy number (13 - 33% of the genome), one or two satDNAs with a medium copy number (1 – 6% of the genome) and the remaining satDNAs with a low-copy number (less than 1% of the genome). Interestingly, the genome size of *Tribolium* beetles generally follows the number of characterized satDNAs. This can be linked to the C-value paradox. The C-value paradox explains that the complexity of an organism does not correlate with its genome size or the number of encoded proteins (Lakhotia 2023). Therefore, the accumulation of satDNAs can lead to larger genomes.

The high number and percentage of *Tribolium* satDNAs per genome is in accordance with similar studies of satellitomes of repeat rich genomes, such as the genome of the insect *Triatoma delpontei* with 160 characterized satDNAs that make up 51.07% of their genomic content (Mora et al. 2023), the genome of the algae *Alexandrium minutum* with 180 satDNAs comprising 17.38% of the genome (Cuadrado et al. 2023) and the Brazilian Atlantic frog *Proceratophrys boiei* whose genome contains 226 satDNA families together spanning 16.87% of their genomic content. However, despite the use of high-throughput genomic sequencing, it is not uncommon to find a lower number of characterized satDNAs per genome in recent literature. To be more specific, the genome of the pygmy mole cricket *Xya riparia* was found to contain only 13 different satDNA families that make up 0.15% of the genome (Khan et al. 2024). Another example is the Aquitanian mole *Talpa aquitania* with 16 characterized satDNAs, including telomeric repeats, representing 1.24% of the genome (Gutiérrez et al. 2023). Also, multiple species of the plant genus *Artemisia* share only 10 identified satDNA families among them (He et al. 2024). Notably, the combination of a few detected satDNAs occupying a large percentage of genomic content can be found in the genome of a *Tribolium* relative, the tenebrinid beetle *Tenebrio molitor* (Oppert et al. 2023). *T. molitor* has only 11 known satDNAs, in combination with the dominant major satDNA. However, its satellitome makes up 28% of the genome, most of it being contributed to the major satDNA. The reason for the huge discrepancy in satDNA number and genomic abundance among species can be explained with the fact that different species contain a different quantity of repeated sequences in their genomes. However, there is a possibility that in some organisms a certain number of the satDNAs were not detected and/or reported due to the unclear criteria of satDNA definition. In this study, the criterion of  $\geq 5$  satellite monomers repeated in tandem in the genome assembly was used, as it was seen as sufficient to form an array. However, in the field of satellite DNA biology the consensus was not yet reached about what truly constitutes the satDNA array. Also, a lot of genome assemblies are still lacking in the satDNA portions, so the more low-copy satellite repeats could still be missed, even with the  $\geq 5$  tandemized satellite monomers threshold.

### **Common characteristics of *Tribolium* satDNAs**

528 newly discovered *Tribolium* satDNAs, together with five already described major satDNAs, share similarities in their general characteristics, the most apparent being the high A+T nucleotide composition of the satellite monomers. The majority of the characterized satDNAs (345 out of 533) have an A+T composition that ranges from 70% to 80%. It is known that the species of the genus *Tribolium* have (A+T)-rich genomes, with the *T. castaneum* genome consisting of 67% (Richards et al. 2008) and *T. freemani* of 67.47% (Volarić et al. 2022) A+T nucleotides. Due to their genomes being abundant in major centromeric satDNA sequences, which are also (A+T)-rich (*T. castaneum* 73%, *T. freemani* 69.9%), (A+T)-rich satDNAs contribute significantly to raising A+T content of the genome overall. It is quite common for satDNAs, especially centromeric satDNAs, to be A+T biased. In fact, satDNAs were first detected because of their differential buoyancy in the CsCl gradient in comparison with the rest of the genome (Kit 1961; Sueoka 1961). The reason for (A+T) inclination could be found in the bending of (A+T)-rich DNA into a curvature which is preferable for the packing of nucleosomes and is therefore complicit in the heterochromatinization of DNA (Fitzgerald et al. 1994). In literature there are countless examples of species with a high A+T content of satellitomes, as seen with three Characid fish of *Psalidodon* and *Astyanax* genera whose A+T composition range in median from 57.9% to 62.1% (Goes et al. 2022), the satellitome of the insect *Rhodnius prolixus* with a mean A+T content of 65.37% (Montiel et al. 2021), and satellitomes of the three plant species from the genus *Passiflora* with a combined median A+T value of 60.9% (Sader et al. 2021). In contrast, satDNAs of the birds of the Corvoidea superfamily exhibit richness in their G+C content that is higher than their total genomic G+C content (Peona et al. 2023). The authors hypothesize that this anomaly can be contributed to G+C based gene conversion that is present in birds.

Most of the satDNAs characterized in this work have a monomer length between 100 and 400 bp, with their medians being 252 bp for *T. freemani*, 303 bp for *T. madens*, 176 bp for *T. confusum*, and 282 bp for *T. brevicornis*. The majority of known satDNAs have monomer length in a similar span (Garrido-Ramos 2017), and it can be speculated that this is due to the packing of DNA into a nucleosome. To be more precise, the nucleosome core is comprised of 147 bp with additional 10 – 90 bp of linker DNA (Richmond and Davey 2003). Centromere specific CenH3-nucleosomes, that are typically associated with satDNAs, have some structural differences in comparison to canonical nucleosomes (Heslop-Harrison and Schwarzacher 2013). The micrococcal nuclease (MNase) digestion followed by CHIP-seq with the CenH3 antibody in rice, revealed that the satDNA *CentO* was wrapped around the nucleosome core with only one turn (Zhang et al. 2013). This finding lead to the conclusion that the evolution of satDNA may be connected with the stabilization of CenH3-nucleosomes by reducing tension and enabling kinetochore formation (Heslop-Harrison and Schwarzacher 2013; Zhang et al. 2013). The satDNAs of *Tribolium* with their median lengths would therefore fit nicely into one or two nucleosomes, enabling efficient packing of the DNA. However, the length of *Tribolium* satDNA consensus monomers spans from 52 bp to 1275 bp. In fact, 50 bp was chosen as a threshold

for defining the sequence as satDNA, as the consensus sequences which had been provided by TAREAN analyses were all longer than 50 bp. The lower length border between microsatellites and satDNAs is not clearly defined, but contemporary authors tend to classify all the tandemly repeated sequences as satDNAs, as seen in the paper analyzing the satellitomes of 37 *Drosophila* species in which authors defined satDNAs with a monomer size as short as 2 bp (De Lima and Ruiz-Ruano 2022). As for the longest characterized satDNAs, they are in accordance even with the known major satDNAs of the *Tribolium* species, as TBREV of *T. brevicornis* is 1061 bp long (Mravinac et al. 2005). More examples of long satDNA monomers can be found in the centromeric repeats of the Welsh onion, *Allium fistulosum*, named AfCen1K and AcCen1K that are around 1200 bp long (Kirov et al. 2020), the cattle species *Bos taurus taurus* with a monomer length of 1419 bp (Melters et al. 2013) and the potato *Solanum tuberosum* whose longest satDNA is 2754 bp long (Gong et al. 2012). Despite the general preference for nucleosome length, the monomers of different satDNAs can exhibit a very wide range of lengths. An open question remains whether the potential secondary and tertiary structures of satellite repeats are more important than the length of the monomer unit itself. This might explain such a wide range of satDNA monomers in eukaryotic genomes.

SatDNAs are often inclined to form complex monomer units. Those complex monomers can be manifested as higher-order repeat organization. 22 *Tribolium* satDNAs were found to have a HOR monomer organization. The most dominant form of *Tribolium* HORs are the dimeric HORs which were detected in the satellitomes of all four species studied. A satDNA with a dimeric HOR structure was also observed in the characterized satellitome of *T. castaneum*. The satDNA in question is TCsat38 and it consists of two subunits that are only 19.4% different from each other (Gržan et al. 2023). Furthermore, the satDNA TCsat18 has an array variant that consists of dimeric HOR units (Gržan et al. 2023). Dimeric HORs, as the simplest form of HOR organization, were also identified in other tenebrionid beetles, with the examples being the insects of the genus *Palorus* (Plohl et al. 1998; Meštrović et al. 2000). HOR-based satDNAs have also been characterized in other non-tenebrionid insects, e.g. the satellite DNA of the leaf beetle *Chrysolina carnifex* that can be arranged into dimeric or even trimeric HORs (Palomeque et al. 2005). Trimeric HORs are less common, but were detected in the satellitomes of the species *T. madens* and *T. brevicornis* in this study. In addition, the hexameric HOR satDNA TmaSat57 was identified in the genome of *T. madens*. However, the HOR organization of satDNAs is not restricted to insects, as the example of the human alpha satellite, which has been extensively studied, shows. Each human chromosome has a unique HOR organization, such as the dimeric HOR structure on chromosome 1 and the 34meric HOR structure characteristic of the male Y chromosome (McNulty and Sullivan 2018). Another type of complex satDNA monomers are repeat units formed by rearrangements such as duplication followed by partial inversion. A good example of this type of HORs is the satDNA TcoSat36 of *T. confusum*, which has a dimeric HOR structure, where one of the subunits is the inverted repeat of another. Inverted repeats are common in the known *Tribolium* major satDNAs. The satDNA MAD2 of *T. madens* (referred to as TmaSat02 in this study) consists of three related subunits, two of which are organized as inverted repeats (Ugarković et al.



1996b). The major satDNA of *T. brevicornis* (referred to as TbrSat01 in this study) has a 1061 bp long monomer unit with a 480 bp long inverted subunit formed by minor direct and inverse duplication events (Mravinac et al. 2005). An even more extreme example of a monomer unit derived by rearrangements is TAUD2, the major satDNA of *T. audax*, which has a 1412 bp long monomer consisting of inversely oriented ~110 bp units (Mravinac and Plohl 2010). Inverted repeats of submonomeric length are common in satDNAs. It is hypothesized that the inverted segments may lead to the formation of secondary structures, such as cruciform and/or hairpins. Experimental evidence suggests that the arrays of the alpha satellite within human centromeres form hairpin structures, which are necessary for the proper functioning of topoisomerase I $\alpha$  (Jonstrup et al. 2008). Furthermore, using crystallography, it was detected that the human satDNA HSat3 forms hairpins by non-canonical base pairings, linking it to possible disease-associated repeat expansion (Chen et al. 2024).

In addition, the monomeric dyad symmetry structure that has been observed in *Tribolium* species, one of the most interesting findings in this work was the observed tendency of some satDNAs to change monomer direction inside the multi-kilobase long arrays, exhibiting imperfect dyad symmetry on the long-range scale. The most notable examples are the TfrSat01/TfrSat03 arrays found in the *T. freemani* genome. On the one hand, inverse orientation of the long arrays could be seen as a passive outcome of structural rearrangements. On the other hand, it is tempting to speculate whether the long-inverted arrays could be forming large DNA hairpin structures, which could possibly contribute to the stability of the genome.

### **Chromosomal distribution of *Tribolium* satDNAs**

Even though the long and highly accurate PacBio HiFi reads used here for preliminary genome assemblies provide invaluable opportunities in characterizing large arrays of satDNAs, they are still not sufficient for the unequivocal determination of chromosomal localization of satDNAs. Tandemly repeated satDNAs still remain difficult to assemble, and there is room for misassembling and errors in chromosome annotation. In this study, the *T. freemani* genome assembly Tfree1.0 (Volarić et al. 2022) assembled into 10 linkage groups corresponding to 9 autosomes and the X-chromosome, was used for the annotation of potential novel satDNAs, so there was a general idea of array number and chromosome position of mentioned satDNAs. However, there are still unplaced sequences that were not included in the official Tfree1.0 assembly. Furthermore, for the three other species, *T. madens*, *T. confusum* and *T. brevicornis*, the preliminary assemblies in scaffold and contig forms were used, so they could not be used to determine the exact chromosomal location of their satDNAs. Therefore, cytogenetical FISH experiments were crucial for determining the position of the most prominent satDNAs in *Tribolium* genomes. Major satDNAs of all the species above were already localized along with their identification in the previous publications (Juan et al. 1993; Plohl et al. 1993; Ugarković et

al. 1996b; Durajlija et al. 2000; Mravinac et al. 2005). Nevertheless, in this study, FISH experiments were performed on the major *Tribolium* satDNAs by using confocal microscopy to recheck their positions and achieve better visualization with higher resolution images. For three of them, the original results were confirmed and their chromosomal position was determined to be in large blocks corresponding to (peri)centromeric regions. TmaSat01, TcoSat01, and TbrSat01 were determined to be located on all of the chromosomes of the complement. The big surprise was the major *T. freemani* satellite TfrSat01 that was not localized on the smallest chromosome of the complement, male  $y_p$  chromosome, by confocal microscopy. Juan et al. hybridized the major satDNA probe to the chromosomes and determined its pericentromeric position, but in their paper, they did not specify whether the satDNA is actually present on all *T. freemani* chromosomes. As the *in situ* figure presented in their paper is not really high-resolution, the conclusion cannot be inferred from it either. Given that the remaining major satDNAs of the *Tribolium* species showed presence on all of the chromosomes, the lack of major satDNA signal on  $y_p$  chromosome is exclusive for *T. freemani*. Additional confirmation for the lack of TfrSat01 on the  $y_p$  chromosome was achieved by the double hybridization with TfSat01/TfrSat03 probes. TfSat03, whose arrays were first identified interspersed with TfrSat01 arrays *in silico*, colocalizes with TfrSat01 on 19 chromosomes, however it is also not present on the  $y_p$  chromosome.

From the earliest days of satDNA research, satDNAs were linked with the constitutive heterochromatin (Yunis and Yasmineh 1971). In the five decades that followed, it has indeed been proven that satDNAs occupy centromeric regions, often even being the main component of the functional centromeres (Hartley and O'Neill 2019). In *Tribolium*, it was determined that the centromere competent DNA sequences of *T. castaneum* and *T. freemani* are their respective major satDNAs and that their chromosomes have a form of metapolycentric centromeres (Gržan et al. 2020; Gržan 2020), so it can be speculated that the large blocks of major satDNAs of the remaining *Tribolium* species also correspond to their centromeres. The positioning of the most abundant satDNAs in heterochromatic blocks of the chromosomes is often seen among other animal and plant species. Some of the numerous examples found in literature include the greater long-tailed hamster *Tscherskia triton* whose satDNA Cen-Taql-S2 was localized on 11 out of 13 autosomes in centromeric regions (Kamimura et al. 2023), the plant species *Ensete glaucum* satDNA Egcen that was found localized in centromeric regions of all of the chromosomes of the complement (Wang et al., 2022), and multiple species of the genus *Palorus* whose major satDNA is present in large heterochromatic blocks of all chromosomes (Meštrović et al. 1998). In contrast to the now outdated dogma that satDNAs only occupy heterochromatic areas characterized by inert recombination, new insights gained through better quality sequencing and genome assemblies show that satDNAs can be located in euchromatin. One of the first examples of detected euchromatic satDNAs are Cast1-9 satDNAs of *T. castaneum* (Pavlek et al. 2015), which were further confirmed to be embedded in gene-rich regions by improved genome assembly generated by long- read ONT sequencing (Volarić et al. 2024). This trend was observed in more insect species, such as *D. melanogaster* and its three *simulans* clade relatives, where

the satDNAs 1.688 g/cm<sup>3</sup> and *Rsp-like* were found to be positioned in the euchromatin (Sproul et al. 2020). Furthermore, in the leaf beetle *C. americana* the low-copy number satDNAs showed a different distribution than the more abundant satDNAs, as they are mainly located in euchromatic regions (Rico-Porras et al. 2024). A similar arrangement was observed in the low-copy satDNAs studied in this work. Cytogenetic analyses confirmed their distinct chromosomal localization, with most of them being localized on only one or a few chromosomes of the complement in the euchromatic regions. The signals obtained by FISH appeared to be more dispersed in accordance with their low genomic abundance.

It is especially interesting how double FISH experiments were able to differentiate satDNAs that are part of the same superfamily, namely the *T. freemani* superfamily A, containing TfrSat03 and TfrSat04, and the *T. confusum* superfamily A, containing TcoSat02 and TcoSat03. The similarity of consensus sequences of satDNAs in question range from 65.52% - 73.04%, but the high accuracy of the PCR labelled FISH probes successfully discerns them at different chromosome locations and there was no false positive colocalization of the mentioned satDNAs. TfrSat03 consists of three subunits, all of which are similar to TfrSat04. While TfrSat03 does not appear on the  $y_p$  chromosome but on all remaining chromosomes of the complement, TfrSat04 was found positioned exclusively on the male sex  $y_p$  chromosome. Except for their joint double FISH experiment, this finding was confirmed by the TfrSat01-TfrSat03 FISH experiment which showed that the two satDNAs do not occur on the  $y_p$  chromosome. Because of the exclusive position on the  $y_p$  chromosome, TfrSat04 could serve in the revision of the Tfree1.0 genomic assembly in which the  $y$  chromosome was not assembled (Volarić et al., 2022), and it can be used as a potential  $y_p$  chromosome specific marker in further studies. Several different species show a similar satDNA pattern, in which there is a satDNA present exclusively on the Y-chromosome. In the plant species *Sea buckthorn*, whose karyotype is similar to that of *Tribolium freemani* with a large X- and small  $y$ - chromosome, Y-specific satDNA HRTR12 was detected, which helped in mapping the Y-chromosome (Puterova et al. 2017). In the species of *Drosophila simulans* clade, several Y-linked satDNAs were detected, each being highly homogenized and spanning only a few kb (Chang et al. 2022). From an evolutionary point of view, the most interesting scenarios are certainly those in which the sex chromosome-specific satDNA shows similarities with its autosomal counterparts, as is the case with the satDNA TfSat04 of *T. freemani*. A similar situation was observed in the plant *Rumex acetosa*, in which  $y$  chromosome specific satDNAs RAYSI, RAYSII and RAYSIII all share similarities with autosomal satDNA RAE730 and all evolved from the same 120 bp unit that underwent different duplication events, likely from recombination suppression of sex chromosomes (Mariotti et al. 2009). Reduced recombination on the  $y$  chromosome could also possibly explain why TfSat04 diverged from TfSat03 and evolved into a  $y$ -specific satellite.

## **The evolutionary aspects of *Tribolium* satDNAs**

### ***Origin of Tribolium satDNAs***

The origin of the majority of newly characterized satDNAs cannot yet be determined. However, some of them point to sequences or elements from which they originated. One of them is the major satDNA of *T. freemani*, TfrSat01. By exploring the *T. freemani* satellitome, the complex satDNA TfrSat02 with a monomer unit of 1106 bp was discovered. Within its 1106 bp sequence, it contains the 166 bp TfrSat01-like element. Phylogenetic analyses revealed that an ortholog of TfrSat02 is present in the genome of *T. castaneum* as TCsat15 and in the genome of *T. madens* as TmaSat03. Together, the three satDNAs (TfrSat02, TCsat15, TmaSat03) were classified into the TribSAT3 orthologous satDNA family. Since there are no tandemly repeated TfrSat01 copies present in *T. castaneum* and *T. madens*, it is logical to conclude that in *T. freemani* TfrSat01 arose from a segment of TribSAT3 sequence. Because *T. freemani* and *T. castaneum* diverged around 14 Mya, it can be assumed that this is also the age of TfrSat01 satDNA which was likely propagated into a highly abundant sequence by amplification burst events. Similarly, a potential source of *T. castaneum* major satDNA TCAST from a longer element was discovered in the *T. castaneum* genome. The authors detected the TCAST-like sequence embedded within a larger element resembling a DNA transposon, but they also found a TCAST-like monomer within the 5' UTR of the CR1-3\_TCa retrotransposon (Brajković et al. 2012). Although the major satDNAs of both sibling species are derived from longer elements, TfrSat01 is specific. In parallel to its propagation into the major satDNA in the genome of *T. freemani*, the initial satellite TfrSat02 continued to exist in a relatively large number of copies.

There are many studies that point to transposable elements as a source of new tandem repeats (Zattera and Bruschi 2022). The majority of satDNAs characterized in this work were found to have partial similarities with transposable elements, most prominent of them being DNA transposons. However, the most apparent connection was discovered with the satDNAs that were grouped into the TribSAT7 and TribSAT8 orthologous family. TribSAT7 consists of satDNAs TCsat23 and TfrSat15. TCsat23 was already identified as being the inverted repeat unit of the subtermini of the Rehavkus-1\_TC DNA transposon, with the question arising whether the origin of the repeat is the transposable element or whether the tandem repeats were captured by transposable elements (Gržan et al. 2023). However, as there are no arrays of TCsat23 longer than 9 consecutive monomers, it was concluded that satDNA originated from the transposon. A similar situation was observed with TfrSat15, in which the majority of the arrays are between 6 and 10 monomers long, with only one array consisting of 29 monomers. But as Rehavkus-1\_TC was already determined as an ancestral element, it could be speculated that the longer array in *T. freemani* formed due to amplification events happening after the speciation event. Regarding the TribSAT8, it consists of TCsat12 and TfrSat25 which both originated from the Rehavkus-like element. Interestingly, in *T. freemani*, arrays of TfrSat25 remained shorter, with only 2-12 tandemized copies, while arrays of TCsat12 underwent bursts of amplification after speciation with the longest array reaching 80.5 kb. Transposons and satDNAs are undoubtedly

interconnected during the evolution of eukaryotic genomes. The fact that DNA transposons can be the origin of satDNAs has been demonstrated in numerous species. For example, in the clam *Donax trunculus*, the high copy miniature inverted repeat transposable element (MITEs) contains tandemly repeated arrays that vary in number of monomer copies (Šatović and Plohl 2013). In the fruit fly *Drosophila virilis*, the DNA transposon named *Tetris* contains tandemly repeated copies of the sequence TIR-220 in its terminal inverted repeats (Dias et al. 2014). TIR-220 is also a satDNA with kb-long arrays in the species *D. virilis* and *Drosophila americana* (Dias et al. 2014). It was found that the most abundant satDNA of the species in the genus *Chenopodium* originates from the *tnp2* domain of the CACTA-like DNA transposon *Jozin* (Belyayev et al. 2020). However, there are more examples of satDNAs derived from class I retrotransposons in the literature. In the legume plant *Lathyrus sativus*, long-reads generated by ONT sequencing were crucial for the detection of the LTR retrotransposon named Ogre which contains short tandem arrays of different satDNAs in its 3' UTRs (Vondrak et al. 2020). Those arrays were proven to be the origin point for nine different satDNAs of *L. sativus* (Vondrak et al. 2021). Centromeric satDNAs So103, So137 and So119 of the cultivated sugarcane *Saccharum officinarum* originated from the CRM element which is a class of Ty3/gypsy retrotransposons (Huang et al. 2021). In the potato species *Solanum bulbocastanum* satDNA Sobo was recently amplified from an LTR of a retrotransposon as it was not detected as tandem repeat in other *Solanum* species (Tek et al. 2005). Furthermore, in the genomes of 56 species of scaled reptiles several different satDNAs were found to be derived from short interspersed elements (SINE) (Vassetzky et al. 2023). Although the origin of satDNAs is often found in different retrotransposons, *Tribolium* genomes are rich in satDNAs that show similarities to DNA transposons. This is not surprising, as DNA transposons represent the dominant fraction of transposable elements in the genomes of *T. castaneum* (Wang et al. 2008) and *T. freemani* (Volarić et al. 2022). Therefore, it can be speculated that the DNA transposons also dominate the repeatomes of other *Tribolium* species, which is an interesting topic for future research.

### **Orthologous satDNAs in the genomes of *Tribolium* species**

Even though major satDNAs of the *Tribolium* species examined in this study share no similarities among each other, several different low-copy satDNAs can be found shared among two or more species. For the comparative analysis, the satellitome of *T. castaneum* was also considered (Gržan et al. 2023), as it is already characterized and could provide informative insights when compared with the other satellitomes, especially with that of its sibling species *T. freemani*. 23 different orthologous satDNAs were found in the five analyzed species, with 11 of them being shared among sibling species, 16 of them among the species of the *castaneum* group and only one of them shared thorough the genus. Notably, the shared DNA named TribSAT1, together with two TribSAT22 and TribSAT23 that contain satDNAs of *T. brevicornis*, can help us in understanding phylogenetic relations within the genus, especially in regards to *T. brevicornis*. Namely, Angelini and Jockusch have

debated *T. brevicornis* classification inside the genus *Tribolium* backed up by an evolutionary study of two mitochondrial and three nuclear molecular markers and classified it into the genus *Aphanotus*. The claim that *T. brevicornis* belongs to the *Tribolium* genus can be substantiated by a closer look at the TribSAT1 satDNA. Not only has the satDNA been conserved among the species with differences consistent with its phylogenetical relationship, but it retained syntenic genomic position in only one array on, presumably, chromosome 3. The flanking regions of the array have also been conserved, with additional confirmation of the phylogenetic relationships. The conserved satDNA suggests that the array and its surroundings were already present in ancestral species and remained frozen for more than 86 My, as the approximation of the basal split within the genus *Tribolium* occurred at that time (Ramesh et al. 2021). This finding is an excellent example of the use of satDNAs in phylogenetic and taxonomic studies, despite their reputation as the most variable parts of eukaryotic genomes. Although it is perpetually considered that satDNAs are not good phylogenetic markers (Bachmann et al. 1993), there are studies showing the importance of satDNA for phylogenetic analyses and determination of taxonomic status. For example, the phylogenetic relationships of the fish family Sparidae were clarified by the highly conserved centromeric satDNA family EcoRI (Garrido-Ramos et al. 1999). Comparative analyses of the satellitomes can be even more informative than analyses of individual satDNAs. In such a way, satellitomes have been used for a better understanding of the phylogenetic relationships between the species of nematodes of the genus *Meloidogyne* (Despot-Slade et al. 2022). The authors suggested that the non-coding part of the genome could be used for deciphering the complex phylogenetic relations among the species where standard markers did not provide definite answers. SatDNAs, defined by repeatome and satellitome analyses, were also used in comparative karyotype studies of the plant genus *Hedysarum* which enabled a better understanding of their common origin and taxonomic status (Yurkevich et al. 2022). New sequencing technologies and improved genome assemblies that make satDNAs more visible to researchers will undoubtedly lead to a more precise application of satDNA evolution as a taxonomic tool.

### ***The evolution of Tribolium satDNAs and mechanisms of their turnover***

The evolution of genomes in the context of the evolution of satDNAs is a fascinating and yet still relatively underrepresented research topic. Species of the genus *Tribolium* are an excellent model for studying the main concepts of the satDNA evolution. The major satDNAs that dominate the genomes of *Tribolium* species show no similarities with each other, with the exception of the sibling species *T. madens* and *T. audax*, whose major satDNAs are based on the same 110 bp long fragment (Mravinac and Plohl 2010). Therefore, the major satDNAs of *Tribolium* species can be used as an example for the satDNA library hypothesis. The presence of the TfrSat01 seed within the TribSAT3 provides further evidence for said claim, as the sequence was present in the ancestral genome, but became amplified in the specific subset of subpopulation that diverged to become *T. freemani*. Differential amplification of shared satDNAs was observed with the grasshoppers of the genus

*Schistocerca*, in which one of the shared satDNAs made up between 0.07% and 6.7% of the total genomic content among 10 analyzed species (Palacios-Gimenez et al. 2020). Low-copy satDNAs of the *Tribolium* genus are more prone to evolving following the concept of concerted evolution. An example of that can be seen with the satDNAs of the TribSAT1 family, which showed that the monomers extracted from the genome of the same species are more similar to each other than to those extracted from different species. However, the evolution of satDNAs in *Tribolium* species is happening as the combination of two concepts, with satDNA library and concerted evolution complementing each other. Even within TribSAT3, which serves as the seed sequence for TfrSat01 propagation, we can see that the satDNAs TfrSat02, TCsat15 and TmaSat03 evolve following the concept of concerted evolution. This is not a new finding, as the literature provides examples such as *Tyba* satDNA from the plant genus *Rhynchospora*, which is found conserved within 68 species of the genus, suggesting the existence of an ancestral satDNA library (Costa et al. 2023). Nevertheless, *Tyba* exhibited clade specific variants which emerged due to the homogenization under the influence of concerted evolution. Furthermore, by comparing the satellitomes of two oedipodine grasshoppers, namely *Locusta migratoria* and *Oedaleus decorus*, the authors commented on the contingent nature of satDNA evolution, through which the satDNA library of ancestral species enables the divergence of satDNA after cladogenetic events (Camacho et al. 2022). Concerted evolution of said satDNAs is in turn lead by the amplification of satDNAs which increases homogenization within species and the divergence between species (Camacho et al. 2022).

Mechanisms by which satDNAs are amplified and homogenized are: unequal crossover, gene conversion, rolling circle replication, and transposition (Dover 1986). In this study, rolling circle replication was explored to determine the co-evolution of the closely linked TfrSat01 and TfrSat03, whose arrays are intermingled. It has been shown that both satDNAs are found in fractions of the eccDNAs. Thus, it can be concluded that the re-insertion of eccDNAs into the non-homologous chromosomes leads to amplification and homogenization of satDNA arrays in the (peri)centromeric regions. The formation of eccDNA has been previously described for the mouse major satellite DNA that is prone to form eccDNAs containing satDNA multimers (Cohen et al. 2006). Interestingly, the long-range organization of mouse centromeres and pericentromeres was recently explored, and parallels could be made between its major and minor satellites compared to *T. freemani* TfrSat01 and TfrSat03 arrays. More specifically, the mouse major satellite is pericentromeric while the minor satellite is present in centromeric regions, with the direction switch occurring in both satDNAs' arrays (Packiaraj and Thakur 2024). Therefore, it might be interesting to investigate the possible roles that eccDNA non-homologous reinsertion could have in the genomic organization of the (peri)centromeric regions of both organisms. Furthermore, the role of eccDNAs in propagation of satDNAs was already explored in *T. castaneum* with the euchromatic satDNAs Cast1-Cast9, where it was discovered that they spread thorough the genome with the help of eccDNAs (Volarić et al. 2024). A similar situation was observed with the euchromatic satDNAs of *D. melanogaster*, where eccDNAs contribute to the evolutionary dynamics of *Rsp-like* and 1.688 g/cm<sup>3</sup> satDNAs (Sproul et al. 2020).

Due to the strong intertwining of satDNAs and transposons in the *Tribolium* genomes, the propagation of satDNAs is likely associated with transposition. This is particularly true for low-copy number satDNAs such as TfrSat15 and TfrSat25, which are mainly represented in the genome as part of larger transposable elements. After discovering the Tc1/mariner miniature inverted-repeat transposon family which they named miDNA4 that contained a satDNA repeat in *Xenopus* frogs, Scalvenzi and Pollet proposed a model for transposon mediated satDNA amplification. Transposons with captured satDNAs undergo transposition, which is followed by amplification of said satDNAs. This cycle continues but as the number of satDNA monomers rises, the transposition rate slows down, eventually giving birth to satDNA arrays (Scalvenzi and Pollet 2014). In *T. castaneum* the propagation of the euchromatic satDNA Cast5 has been linked to the non-autonomous Tc1/Mariner DNA class II transposon due to its proximity to the Cast5 arrays (Pavlek et al. 2015; Volarić et al. 2024). The DNA transposons *Cg\_HINEs*, classified into the *Heletron* superfamily, have been shown to carry tandemly repeated sequences throughout the genome of the Pacific oyster *Crassostrea gigas*, making them an efficient propagation tool for satDNAs (Vojvoda Zeljko et al. 2020).

### **Potential functions of *Tribolium* satDNAs**

SatDNAs dominate the genomes of the species of the genus *Tribolium* so the question arises of their potential roles. As for *Tribolium* major satDNAs, their genomic abundance and position in centromeric heterochromatic blocks indicate their centromeric function. In the case of *T. castaneum* and *T. freemani*, it has been already confirmed that TCAST and TfrSat01 are associated with the centromeric histone variant CenH3 and thus are part of functional centromeres (Gržan 2020; Gržan et al. 2020). Since other major satDNAs follow the same pattern of organization in pericentromeric and centromeric regions and have the same properties such as the A+T composition, their function as centromere competent sequences can be assumed. However, novel findings on the long-range centromeric organization of *T. freemani* explored in this study revealed that arrays of TfrSat01 are interspersed with satDNA TfrSat03. This organization closely mirrors the organization of the TCAST arrays and the Cast7 arrays (Volarić et al. 2024). Notably, even though TfrSat01 and TCAST share no nucleotide similarities, TfrSat03 and Cast7 are orthologous satDNAs. Therefore, the question arises whether TfrSat03 and Cast7 really are actually centromere-competent satellites, while the major satDNAs TfrSat01 and TCAST only form the centromere scaffolding. On the other hand, TfrSat03/Cast7 orthologues have not been found in other *Tribolium* satellitomes, even in the most closely related *T. madens*. Still, in the *T. madens* genome the major satDNAs TmaSat01 and TmaSat02 also exhibit a similar pattern of organization, with TmaSat02 being embedded in longer arrays of TmaSat01 (Durajlija et al. 2000). The genome proportion of all the satDNAs mentioned is also mirrored, with TCAST, TfrSat01 and TmaSat01 being an order of magnitude more abundant than the second most abundant satellites in their genomes. The mouse genome is a great example of a minor satellite with a lower abundance being centromere competent, while the major satDNA occupies the



pericentromeric region (Packiaraj and Thakur 2024). Thus, it is evident that the most dominant sequence is not necessarily the functional one. Moreover, those findings provoke the question of whether the nucleotide sequence is really the most important factor for centromeric function, or whether the true functionality could also reside in the structure of the arrays. However, high abundance and chromosomal location of major satDNAs can be discussed in the context of speciation and reproductive isolation among species. Once again, the sibling pair *T. castaneum*/*T. freemani* represents the best test field to clarify these questions. Comparative analysis of the two closely related species, which can even hybridize and produce sterile offspring, demonstrated that their coding regions are similar (Volarić et al. 2022). Since the major satDNAs provide the biggest genomic differences between the two genomes, the question arises whether they are responsible for the postzygotic reproductive barrier between the species. It has been shown that different satDNAs in *Drosophila* hybrids are responsible for the lagging of chromatin during embryogenesis, leading to improper mitosis (Ferree and Barbash 2009) and lead to an improper assembly of chromocenters (Jagannathan et al. 2018; Jagannathan and Yamashita 2021), proving that satDNA divergence between closely related species can cause reproductive isolation. Future research into the role of major satDNAs of *T. freemani* and *T. castaneum* in the reproductive isolation of the two species may therefore provide additional insights into how the evolution of satDNAs shapes the evolution of the genome as a whole. The potential function of newly discovered low-copy satDNAs is even more enigmatic. The satDNA TribSAT1, which was conserved across all the analyzed *Tribolium* species could potentially have a more prominent function as it did not succumb to evolutionary pressure and remained highly conserved, as well as its genomic surroundings. The remaining low-copy satDNAs that are scattered across the euchromatic regions of the chromosomes could possibly be important regulatory elements for gene function, or just provide suitable genomic organization and secondary structures for proper genomic functioning. Moreover, some of them might even be more energetically harmful to the genome than they are functional, so that they could be lost in future generations without endangering the persistence of the genome. In contrast, as the satellite library hypothesis suggests, some of the newly discovered low-copy satDNAs could serve as seed sequences for potential novel dominant satDNAs in the yet unknown daughter species of the contemporary *Tribolium* species. A specific pool of sequences that could take over certain tasks such as centromeric function, in the event of sudden unfavorable environmental conditions and the resulting genome instability, could be a valuable genome “safety net”. Although this explanation is highly speculative, it is in line with the still disproportionate relationship between the high abundance and still inconsistent functionality of satDNA sequences in eukaryotic genomes.

## 5. CONCLUSIONS

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1. The satellitomes of the four *Tribolium* species were characterized. Each of the satellitomes analyzed consists of more than a hundred satDNAs, with 135 satDNAs identified in *T. freemani*, 124 in *T. madens*, 108 in *T. confusum* and 166 in *T. brevicornis*, bringing the total number of *Tribolium* satDNAs catalogued in this work to 533.
2. The *Tribolium* satellitomes follow the same pattern, with one major satDNA being dominant and high-copy-numbered, one or two satDNAs being moderate-copy-numbered and the remaining satDNAs being low-copy-numbered. The major satDNAs comprise megabase-long arrays and are located in large heterochromatic blocks in the (peri)centromeric regions, while the low-copy-number satDNAs are scattered in the discrete arrays on fewer chromosomes.
3. Despite the differences in abundance, all satDNAs generally have a high A+T composition and most of them tend to have similar monomer lengths. A tendency towards higher-order-repeat structure of monomer units was observed in a certain number of *Tribolium* satDNAs. In addition, some satDNAs were found to exhibit dyad symmetry in their monomer structure, but also in satDNA arrays, possibly leading to DNA hairpin and/or crucifix structures that may be important for genome stability.
4. Based on comparative analyses of the *Tribolium* satellitomes and the orthologous satDNAs found, it is concluded that major satDNAs and low-copy-number satDNAs evolve according to different evolutionary concepts. The evolution of major satDNAs is more consistent with the “satDNA library” concept, whereas low-copy-number satDNAs are more likely to be influenced by concerted evolution. Of the mechanisms responsible for satDNA turnover in *Tribolium* genomes, rolling circle replication and transposition may be the most influential.
5. Similarities in the long-range organization of the centromeric regions of the sibling species *T. castaneum* and *T. freemani* suggest that the secondary structure may be more important for the centromere function than the nucleotide sequence itself. The complete difference in the nucleotide sequences of the major satDNAs and the similarity between the less abundant orthologous satDNAs in the centromeric regions of *T. freemani* and *T. castaneum* also calls into question the previous assumption that the most abundant satDNAs are key components of functional centromeres.
6. Since the major satDNAs represent the largest genomic differences between *Tribolium* genomes, it is tempting to speculate on their possible role in the postzygotic reproductive barrier between closely related species.

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## 7. SUMMARY

Ocjena rada  
u tijeku

In addition to single-copy sequences, eukaryotic genomes consist of a large proportion of repetitive sequences. These are often dominated by satellite DNAs (satDNAs), tandemly repeating, non-coding sequences that are mainly located in heterochromatic regions of chromosomes. The tandemly repeated organization in combination with their high genomic abundance makes the study of these sequences extremely difficult and challenging. In the past, satDNAs were identified using classical molecular methods, and in this way only the most abundant satDNAs in the genome could be detected. Furthermore, satDNAs are often omitted from genome assemblies because long repetitive sequences are difficult to assemble correctly. The recent development of new generation sequencing technologies, including long and highly accurate sequencing with PacBio HiFi technology, and the accompanying development of bioinformatics tools have enabled the detection of numerous satDNAs in eukaryotic genomes. Given the large number of satDNAs that were discovered, the term satellitome was introduced in the field of satDNA biology, encompassing all satDNAs within the genome.

The flour beetles of the genus *Tribolium* are cosmopolitan pests that occupy food storage facilities. A representative of the genus *Tribolium*, the red flour beetle *Tribolium castaneum*, is also frequently encountered in laboratory research worldwide. It is known that the genomes of *Tribolium* species are dominated by the major satDNAs, which can account for up to 60% of the total genomic content. Interestingly, the major satDNAs of these species do not show similarities in their nucleotide sequences. The species *T. castaneum* is the only one among them that has, in addition to major satDNA, a defined satellitome which counts a total of 57 satDNAs. The aim of this work was therefore to characterize the satellites of four additional species of the genus *Tribolium*, namely *Tribolium freemani*, *Tribolium madens*, *Tribolium confusum* and *Tribolium brevicornis*.

Satellitomes of species from the genus *Tribolium* were characterized using a combination of experimental and bioinformatic methods. Firstly, the genomes of the four species were sequenced using two sequencing technologies. The first technology is Illumina sequencing, which provides short 151 base pairs (bp) reads. These reads were used for *de novo* definition of potential satDNAs using the bioinformatics program TAREAN based on the de Bruijn graph clustering. Sequencing with PacBio HiFi technology yielded long and highly accurate genomic reads that enabled the bridging of long satellite arrays. The aforementioned reads were used to generate preliminary genome assemblies, and in the case of the species *T. freemani*, a high-quality genome assembly. Potential satDNAs obtained through the TAREAN program were annotated to genome assemblies and in this way 533 satDNAs were defined in the genomes of four species of the genus *Tribolium*. The chromosomal localization of the most prominent newly defined satDNAs of each species was experimentally determined by fluorescence *in situ* hybridization (FISH). The results of the FISH experiments showed that the major satDNAs are localized in large heterochromatin blocks of pericentromeric and centromeric regions,

suggesting their possible centromeric role. The signals of the low-copy number satDNAs are found on a smaller number of chromosomes have a much lower intensity and are often located in euchromatic regions. Since some satDNAs are specific to certain chromosomes, they could be used as chromosomal markers in future research.

Furthermore, comparative analyses of the four satellitomes described in this work and the satellitome of the species *T. castaneum* identified 23 orthologous satDNAs in congeneric genomes. The distribution of orthologous satDNAs corresponds in principle to the taxonomic position of the species within the genus. One satDNA was discovered within all the studied species, which can be considered ancestral, and potentially a diagnostic satDNA for the genus *Tribolium*. From an evolutionary point of view, based on the changes in nucleotide sequences within the orthologous satDNAs, it is concluded that low-copy satDNAs preferentially evolve according to the principles of concerted evolution. The evolution of major, species-specific and dominant satDNAs could be explained by the "satellite library" theory. In the case of the major satDNA of the species *T. freemani*, it was discovered that duplication of the major satDNA from the ancestral "satDNA library" does not have to mean duplication of the entire existing repeat unit. Only part of the sequence can be duplicated, expanding the current understanding of the "satDNA library" concept. Regarding the mechanisms leading to satDNA propagation in the species of the genus *Tribolium*, it has been experimentally confirmed that the rolling circle mechanism mediated by extrachromosomal circular DNA (eccDNA) is involved in the spread of highly represented sequences. Analysis of the organization and similarity of the low-copy satellites to transposable elements led to the conclusion that transposition may be the most important mechanism in their evolution. A comparative analysis of the satDNA composition and organization of the centromeric region of the closely related sibling species *T. castaneum* and *T. freemani* revealed that although the major satDNAs of these species lack similarity in nucleotide sequences, they share a similar type of organization based on directly and inversely oriented satellite segments. This suggests the possibility that for centromeric function, the secondary structure of the centromeres is more important than their sequence. In addition to the fact that the sequences of the major satDNAs lack nucleotide similarity, the discovery of low-copy orthologous satDNAs in the centromeric region of the sibling species raises the question of which satDNAs are truly centromere competent.

This work laid the foundation for future research of the function of satDNAs, particularly in relation to the evolution of the genus *Tribolium* and the role which centromeric satDNAs might play in the postzygotic reproductive isolation of species.

## 8. SAŽETAK

Ocjena rada  
u tisku

Eukariotski genomi sadrže uz jedinstvene sekvence također i velik udio ponavljajućih sekvenca. Među njima često dominiraju satelitne DNA (satDNA), uzastopno ponovljene, nekodirajuće sekvence koje se prvenstveno nalaze u heterokromatinskim regijama kromosoma. Uzastopno ponovljena organizacija u kombinaciji s visokim udjelom čini istraživanje ovih sekvenca izazovnim i zahtjevnim. U prošlosti su se satDNA identificirale pomoću klasičnih molekularnih metoda te se na taj način moglo detektirati samo najzastupljenije satDNA u genomu. Uz to, satDNA često su izostavljane iz sastavljenih genoma zbog nemogućnosti pravilnog sastavljanja dugih ponavljajućih nizova. Recentni razvoj tehnologija sekvenciranja nove generacije, uključujući dugo i precizno sekvenciranje pomoću PacBio HiFi tehnologije, kao i popratni razvoj bioinformatičkih alata, omogućio je detekciju pregršt satDNA unutar eukariotskih genoma. S obzirom na velik broj satDNA koje su se počele otkrivati, u području biologije satDNA uveden je pojam satelitom, koji obuhvaća sve satDNA unutar genoma.

Kukci brašnari iz roda *Tribolium* kozmopolitski su štetnici prisutni u skladištima hrane. Predstavnik roda *Tribolium* kestenjasti brašnar *Tribolium castaneum*, ujedno je često prisutan i u laboratorijskim istraživanjima širom svijeta. Poznato je da genomima vrsta roda *Tribolium* dominiraju glavne satDNA koje mogu činiti i 60% ukupnog genomske sadržaja. Zanimljivo je da glavne satDNA ovih vrsta nemaju sličnosti u svojim nukleotidnim sljedovima. Vrsta *T. castaneum* jedina je među njima kojoj je uz glavnu satDNA definiran i satelitom koji ukupno broji 57 satelita. Cilj ovog rada bio je stoga karakterizirati satelitome četiri dodatne vrste iz roda *Tribolium*, poimenično *Tribolium freemani*, *Tribolium madens*, *Tribolium confusum* i *Tribolium brevicornis*.

Satelitomi vrsta iz roda *Tribolium* karakterizirani su kombinacijom eksperimentalnih i bioinformatičkih metoda. Početni korak bio je sekvencirati genome četiriju vrsta upotrebom dvije tehnologije sekvenciranja. Prva tehnologija je sekvenciranje Illumina kojim su dobivena kratka očitavanja duljine 151 parova baza (pb). Ova očitavanja korištena su kako bi se *de novo* definirale potencijalne satDNA upotrebom bioinformatičkog programa TAREAN baziranog na klasteriranju putem grafova de Bruijn. Sekvenciranje pomoću tehnologije PacBio HiFi produciralo je duga i vrlo točna genomska očitavanja koja su omogućila premošćivanje dugih satelitnih nizova. Spomenuta očitavanja korištena su za sklapanje preliminarnih genomske sklopova, a u slučaju vrste *T. freemani* i visoko kvalitetnog sklopa. Potencijalne satDNA dobivene programom TAREAN anotirane su na genomske sklopove i na taj je način definirano 533 satDNA u genomima četiri vrste roda *Tribolium*. Najprominentnijim novodefiniranim satDNA svake vrste eksperimentalno je određena kromosomska lokalizacija pomoću fluorescencijske hibridizacije *in situ* (FISH). Rezultati eksperimenata FISH pokazali su kako su glavne satDNA smještene u velikim heterokromatinskim blokovima pericentromernih i centromernih regija, što sugerira njihovu potencijalnu centromernu ulogu. Signali niskoizostupljenih satDNA pak nalaze se na manjem broju kromosoma, znatno su manjeg intenziteta i često prisutni u eukromatinskim regijama. Budući da su neke satDNA specifične za određene kromosome, u budućim istraživanjima mogle bi se koristiti kao kromosomski biljezi.



Nadalje, komparativnim analizama četiri satelitoma opisanih u ovom radu te satelitoma vrste *T. castaneum*, identificirane su 23 satDNA koje sadrže ortologne satDNA u kongeneričnim genomima, pri čemu distribucija ortolognih satelita načelno odgovara taksonomskom položaju vrsta unutar roda. Otkrivena je također jedna satDNA koja je prisutna u svim analiziranim vrstama te se može smatrati ancestralnom i potencijalno dijagnostičkom satDNA za rod *Tribolium*. Evolucijski promatrano, na temelju promjena nukleotidnih sljedova unutar ortolognih satDNA zaključuje se da niskozastupljene satDNA preferencijalno evoluiraju sljedeći principe usklađene evolucije. Evolucija pak glavnih, vrsno-specifičnih i udjelom dominantnih satDNA mogla bi se objasniti teorijom „satelitne knjižnice“. U slučaju glavne satDNA vrste *T. freemani* otkriveno je da umnožavanje glavnog satelita iz ancestralne „satDNA knjižnice“ ne mora podrazumijevati umnožavanje cijele postojeće jedinice ponavljanja, već se može umnožiti samo dio sekvence, čime se proširuje dosadašnje poimanje koncepta „satDNA knjižnice“. U kontekstu mehanizama koji dovode do propagacije satDNA kod vrsta roda *Tribolium*, eksperimentalno je potvrđeno da mehanizam kotrljajućeg kruga posredovanog ekstrakromosomalnim cirkularnim DNA (eccDNA) sudjeluje u širenju visokozastupljenih sekvenca. Analizom organizacije i sličnosti jednog dijela niskozastupljenih satelita s transpozonskim elementima, zaključeno je da bi u njihovoj evoluciji transpozicija mogla biti mehanizam od najvećeg značaja. Komparativna analiza satDNA sastava i organizacije centromernog područja između blisko srodnih sestrinskih vrsta *T. castaneum* i *T. freemani*, otkrila je da iako glavne satDNA ovih vrsta ne dijele sličnost u nukleotidnim sljedovima, pokazuju sličan tip organizacije temeljen na direktno i inverzno orijentiranim satelitnim potezima te sugeriraju mogućnost da je sekundarna struktura centromera važnija za njihovu ulogu nego što je to njihova sekvenca. Također, nastavno činjenici da sekvence glavnih satelita ne dijele nukleotidnu sličnost, otkriće da u centromernom području sestrinskih vrsta postoje ortologne satDNA manjeg udjela otvara pitanje koji su sateliti uistinu centromerno kompetentni.

Ovim radom postavljeni su temelji za buduća istraživanja funkcije satDNA, posebice u kontekstu evolucije roda *Tribolium* i uloge koje bi centromerno pozicionirane satDNA mogle imati u postzigotnoj reproduktivnoj izolaciji vrsta.

## 9. SUPPLEMENTARY MATERIALS

Ocjena rada  
u tisku















<b>TmaSat21</b>	AGAGTAAAGGACAACTACAAAAACACTATAAACTATAAAATAAAAAAACTACAAGCAAATCAATTTCTCAGACTTCGTAGTTACAGTTTTGAAATGACA AGACTGGAAATTTTCAACAAAAATAAAAAAATGTTTATTGATCATCTTTAATTAATAAATTTAAAAAACTCAATTTTTGTACTCTTATCGAACATCT AGTTTATAAAAAATGAGCTTTTATTAAGAAATTTAAGTTTTTATGACTATAAAATAACTAACGAGACACTAAATTTAATACGAAAAAACTGTAACCTAGAGA GTTAAATTTGAAATTAATAAGAAATCGTATATTATGTTATTAGGAGTAAAGTGAAGTTAAGAGACGAAGCTGAACATTTTAAATAATTCAGCTGAGCCACATGACAA ATAACATACTATTTAATCCCAAACGTATTAGATCG
<b>TmaSat22</b>	TCGTAATAAGGACAAAAACACTCTAAAATGTTTTGAAATGAGTGAATTTGAAAAATTTAAAAATGGGAGTTCACCCAGAAAAGTTTTAATGAAAAACA AACGAAAGGCACAGCTTCAGAGACTTAAAGGAGAGCTTTGTGTTTTTTTT
<b>TmaSat23</b>	CAAAATTTCTCCTCCTTTTTATCTGTAATAAATAACAAACCTAAATCATAGAAATCACAAAACAATAGACAAAACAGTGGATTGTACAAAATTAGAGAAATATT TGAATCTTTGAAAAATCTCGTGAGAAAAATTTAAGGTTTCAAATGTTGCCAAACCTTAAACTTTGAACTTACAATGTCCAAAACGCACAAAATTTTATAATTTAC CTTTTTGACTTCAAAAAATAAAACTTGAATAGAAATGAAAAATTTAGTTTTATGAAATGATTCTCAAATACAGAAATTTTCAAAATAAAATGATAAAAAATTT TCAAGCTCAAAAAACTTATAGTCTGATTTTTAAGTTGTAGAATCTCATAATTTACATATTTTTTTTGATTAAAAAACTTAAAGTTGAAGTTCTCAGCTAATGTTT TACTTTTTAAGTTTTAAACACCGCTAATTTCTAATTCACTTTTTTCAAATTTTAAAGTTTTTCTAGGTTATTTTGA
<b>TmaSat24</b>	GCTTCTGAATATCTATAGTTTTATCTTAAAAATGTTAAGTTACTAAATCAATTAATTTTTGAAAAAATAACTTGTATTAATTTCTGCTTATCTTTGAAAA AGTATAAAAGTGTGAAATCCAGTAAAGTTGTTGTTGCAAAACAACTCACCTTTACGGCAAAATTTCACTAATGATATAGAGTCCAAAAGTACTTCTTACAGACA ATTTTAGGCTACTTTATTTGTTATATCAGAAAGCAATAAAACCTCTAATTTTCAAGAAAGTTTATTTTCAATTTTACATAATTTAGAGCTTCTAATTTATC TATAGTTTTAATTTAATAAATTTGGATAACAGCTTGTCAATTTGATTAATTTGTGTTTTGTAGGAAAGTTTACAAGTGACAAAATTCAGCCATTTTTGATAAAAT TAAGA
<b>TmaSat25</b>	GTGATGAATTTTTTAATAACTGCTTTACTTCGAAATACAGAAACAAAAGAAATAATATAAACTTTTGGAAAAAATTTGAAATAGTATATTACATGCATGTTG GAAAAGTGCATATTTGAGAACTGACATTTCTACTATTTATTGACATTTTACAACAAATGAGTTTGGCGACCTCAAACGAGAAAAACAAAATCTCAAAAAA AAGTAAAAAAGCTTATTACGAGTTTATTTTTTTTTG
<b>TmaSat26</b>	TTCTTTTATGTCACCTTCTAGTTTTCTTCAAAGTATATTTTCTAAATCTAGACCTTTTGGCCGAGGCTCGAGCCCGGAAAAATTTTAAATTAAGAATACATA TTTTTAAAAAGATAAAAAATAAATGCAATTAATAGCTATTCTACATCTAATAAAAAATTTCTACCTCTCAAATGATTTTATCGTTCCGTTAAAAAGACTATTTTT TATTTTCTACTGTTTAAAGCTTGATAATTTATCTCAATTTCTACATCACTGTTTTTGTAGTTAGTGAAGAAATTTACTGTTTTCTATTTTTCTTTAATTTGTAA TTTTTTTACTTATGTCATTTCTAGTTTTCCCACTAAAGTATGTTTTCTAAATGTTTTTATTTTCTCCAAATTTTTTATTTCACTCAACGCTCTACTCTCT CAACTCTGTTTTTTTTTTTTTTTTTTTATTTTTCATAAAAAATTTGTTCTGATTTCTTTTTTACTTTTCTTTTTTGAAGTCCGCCATCAAAGCAACAGCTGATAATTTACCTC AATATTTCTGCTATTATCAGTTTTCAATAGGTATTTTGTTCCTAAAACACTTCAAACCTCTATTATGTACAAAATAAATAAGAGATTATTTTCAATTTAATTTTGA CATT
<b>TmaSat27</b>	TAAACAAATTCGGTTAAATAAAAAACGAGTGATTCAATTACGTGGGGCTATGGGCCAGCCGTTTTCTTTCCGCTCTAGAGATGCGAAAAACAACGAATTAAGC CCCCAGTGCATAATCCGGAAGATT
<b>TmaSat28</b>	CCAAAAACAAAAACTCATAAATGTTTAAATCTTTTTCACACTTTATCTTCACTTTCTTATTAATAAATAGTCAAAAAAAGAGTACCAACAACACTTTCTTACCT CAAACGCCAAAAACCCAAATTTGAAAAATTAACGCTTCAAGCACTAATTTCAAAACAATCTTAAATTTCCCAAATTTTAAAAATGTTCTAATAATTTTTGGG TACTTTTTCAGAAAAACGGTACCAAGTCTCAAATCAATTTTGTACCTAAACCCCTTAAATTTGAGAACTGAAATCTTAAATTTGAGAACGTAAAAAAGTACGATCTCTCACTTTCCA AAATATTACTCAATTTTCAAATTTTGAATAATTTCTCCATGTTACTCAAGTCCAAAAAGGCCATTTTTTCAAACAAAAAT
<b>TmaSat29</b>	TTGCAAAATTAAGTCCAAAAGCGTTTTTTCGCCTGTTTTCCACCAAGAAAACACTCAGTGTGAAATTTTTTCAAACATAATAACAGAAAACGCAAAATTTTCTATCTGT CTAAGCAGTTTTTCCACCAAGAAAAACACTAAATTTTTAAACAGGAACATTTCTCGATGAATAAATATTAAATACATTTTATCGACTTGTGCAAAATTTAGGAAATTAAT TAACCACGTTTCCAAAT
<b>TmaSat30</b>	CGAAGAAAAACAAAAACCAACATTTTTAAAGTTAAAGTAAACGAATCAGAAAAATAAAAATATCGGCATTTTTTAAATACAAGGCAAGGCGAGTATAGAAATATA TACAAGTGCATAATAGTTTTTATGAGTCTATATCAGCTTTGTATAATTTAAAAAAATTAATAATTTAAAAAATGATTTTTTGTTCAAAAGCATAATTAAGAAGAAC AAATTTTTGTTATCAGATTCAATTAAAAAAAATTTGCAAAATCGCAAAATTTGTACATTTCCAGACAACITTCAGGTTTGAAGACTTTTCTCATTTTTTAAATA TG
<b>TmaSat31</b>	TAAAAAAACACTTCTATTGAAACGCAATTTGAAAAAAGAAATAAGGCAACAAAAAAGAGTAGTTGCAATTTTATGAGGAAACAAAGGATTTTTTATTTAATAA TGAAATTAATAAATAAATCAACACTTCAAGCTTCAAAAACTCAAGTTTCAATTTTAAAAAGTCTATAGTCGCTGTTTTAAAAACAATAAATTTGTCAGTTTCGGTTTT TAAATTTGTTAAAAAATTTTAACTTTTAGCAAAAAAACTGCTTTAGGAAATTTGTAGAAAAAGTGT
<b>TmaSat32</b>	ACACAACCTGAGTAAAAATCGCTAAATGAATCGCTTGGGCTTTTTGTAGTTTTTCAACGAAAAAATAAATTTTGAACATTTATTCGTTAAAAACAAACAAAAAT TTATCAAAATTAACAGAAATAATATATTAGTATTTTTAAATGTTTTAACCACGTTTTTAAATAAAAACTAAATTTTTTGAACGGAATAATTTGACTTTTCAA TAGTTTTAAATTTGATGTTTTATGTCATCTGTCAAGATGTTTTGGAGTTTGTATTTGACATCTATGTTATTTGACAGGTTGCAAAATTTGGCGGGAAGTAAATTTT ATTAAAAAATTTGATGGAATAAAGTACCAAACTTTTTACGAGATTTC
<b>TmaSat33</b>	GAAATAACAAAAATAGAGCTAAATGAAGGTAGAGTTGGAATAAAATCAATAAGATAGAGCACAATAAATCTCAATTAATAAATTAATCACTTCAAAAATAGAAATGA GCTACAATAGAGCCA
<b>TmaSat34</b>	TTTTCTGCGGAGTATTTAACTGGTTATACATGTTTTTGTCTCTATTTATGTCAAAAAATCGTATTTTTAAGATTGTTACTTAAGATAACGTCAATTTCCGGCAAT TCAAAAAATTTCCGGTAAAAATTTGGTCTCAGAGCAATTTACTCGTTTTTTAGGAAATTTCAAAAATGTAACAAAATAAACGTTTCACTAGATCTCAAAAACCTG
<b>TmaSat35</b>	TATTTAAAAAATAATAGAGGGAGATATAAAGAAAGATGGAAAAAAGTTAGACTTTTTCTAGATTTTTAAAAAGTGTAGGAACAATTTAAAGAAAGATCTAGA AAAAAATTTAAATAAATCTAGGGAGAAAAAG
<b>TmaSat36</b>	TTCTATAAAAAATTTAAATCTAATATAGACTCAATTAACAATCTATAAGCAAAATTTTTAGTTTTTGTGTTTTAACATTACTTTTTTAAAGTTTGAATTAAGGCTA AAATTTAATTTTTTAAAGAACTCCATTTCAATAGTATAAAAACTGCAACTTGTGAAATTTGACTTAGACTCAATTTCAATCTATAAGACAATCTTTGTCAAATTTGTGG ATTTTAAACATAATTTCTGTTTCAATTTTGAAGTAAAGTACTAGAAGATAATGTTTTCGAGTGAAGTCAATTTTCAG
<b>TmaSat37</b>	GAATTTTTAAAGGTTTTTCCCGGAAAGAAATCAAAAAATTTGAAAACTTTGCAAAACATATCGAAATTTTTTATATTTTCTTAATAAAAAATAACAACTGACTAAC GAGTTGTAATTAATTTGGAAGAAAAAGCTTTTTAGACTACGTAATGATCTCCTAAAAAGCTTTTCTAATAAATTTAATAAAAAAGTACAATCACGGATTTTTTTTT TAAACTAAATTTGAAATTTATCTCTGGAATGCTATTTTTGTCTTATTTTTGTGAATTTAACGCATTTTTTAGCAAAAACTTAATAATCAAAAAAGTTTTTAAAGCAAGA TAGAAAAATTTGCAAAATTAAGAACGAAATGTGTT
<b>TmaSat38</b>	TTTTTTAATGAATTTGGCGTTTTGTAGTTTTTTATTTATTTATACATTATGATGACAGATTTTTTTGTAATATCCACATTTTTAAGAGCGATAAAAAATTTACTGCTGCAAA ATTTTTATGCAATATCTGGAATAATTCATTAACAGGTAATTTTTATGATAGTTTTAATTAATAAACTGATCAAATAAATGAAGAAATAGTTTCTGTTAGCTTGT TGAAGCTAAAAATTTCTTATTTTTCTTTTGTAGTTTTTTTTCAGGCTCTAATAATTTGTCAAAAAGAGAAAAATGTAATAAATTTATCAGACTATAAAAA TAAAAACCAACAGTTTTTCCAAATAATTAACAATAACAGAGAAACAACTGCTGATACCTTTAGATTATTTAATAAAAAATCACTGAAAAAAGTTAATAATTTAATAAT TATTATTAATAATCTGAAAAATTTCAATAAACAGGTAATTTTATGATTTTATGATATTAATAAAGCTTTTGAAGCTCTTTCGAAAAATTTTTTTTCTTTGTTTT TTGTTTTTTTCTGCTCTTTATGAGTTATGACATAAGCAGCTTCTTTGAGTTTTTCTCTCATGATTA
<b>TmaSat39</b>	AAAAATGTGCTCAAAAGGAAGAAATACCCTATTTTTGTAATTTTTGGCTTATCTTCAACGCAATTTTGAATAAATTTGCGTTTTAGCTGAAATTTGCTTTTTGGAC ATTGTTAGTAAAAAATAAATGAATTTTTCACCAAAATTTGGTATTTTTCAATTTTAAAGAAAAATTTGACGAAATTTTCAATCGGGATTTACCAATTTCACTAATTTAGT AGTACATACAGAGTATTTTTTAAATCGACCGAAATAAACGAAATAACACTATTTGAAATTTAATTTTGGCTTTGATTTAATTTAATAATTTTAACTGTTAAAT TTTTTATCTC
<b>TmaSat40</b>	CTAATCACTTAGGGGAGACTGACCTCTAAGATATTAAGGAAAGTAAAACTTTTTGAAAAACAACATGTTTTGCGCCAAATTTGGATTTCTTTGAAATGATTCAATTA AAACGTTAGATTTTCAATAACATAAGCAAAAAACGACGAAATAACCGAAAAAGGTTTTTTGTAGATAAATGGGCTTGAACAAATGAAAAATAACACAAAAATTTA AAAAACAGACAAATTTTCTTAAAAAATACAGATTTGCAAGCAGATTTGGCAATTTCTGTTCCATTTAAAAAATCAAAAAGTACGATGTAATAAATCAAAATC AAATGAAAAAATA
<b>TmaSat41</b>	TTATATTATTGGCGTTTTTTTTGTATTTAGAAGCTAAAAACTTAGAGACGTAGAATTTGAATCTTAAAGTTTGTATTTTCTAGATTTCAATAGTCTATAACAAAAATTT GTTTGAATCTCAGAAACAATAAGAAGCTGAGTGTGAGAAAGATAATTTAAATTTGACAAAGCTGGAGAACTTGAATAATCTAGAAATTTTCAAAATTTTAACTCAC AGTAATTTCAAAATTTAGTACTGAATTTAGAAATTCGCAATTTCAATGAGAAATTTGAAAGATTTTTTCAAAACTAAAACTACTTAAATAGGTTTAAATTTCTAGAAT TGCTAATTTCCGAGAGTAAAAATTTAAAAATTTAGAATGGAAGACCAAAATTTAAAAATTTTAGTACTTATCA
<b>TmaSat42</b>	CGGTGAATAATAACAAAGATAATGAAAGAAAAACAAGATAACCTTTGGAATAGAATTAATAATGGAACCGTTTGAAGAAAGTCAATTTTAAAGAACTAGAAAGATC TTAGAAAAATTTAAAAAATAACATAAATTTGTTAAAGTTAAGATCTTGGTGAAGTTTTGAAGCTTAAAAAACAACATCAGAAATGTTGAAAAATAGTGTAAAAATCTTG



	TTTCTCATGATTATCTGATTTTTCAAAATCAAAAATCTTCTAATTTGTTAAACAATGAACAAAAAAGACCCTACTTGCTTAAATTTCTATAATTTTTGTAAATTC TCATAATTTCTAGTGATTTTCAAATCTGTACTGTACTCTTTTTGGTTGATTTCTCATGATTTTCTGATTCTGTGCAACAACCTGGACAAAAA ACATTGAAATGAGCAACTGATTTTTTCTCCTTAAACAATAAAAAAAGCAAAATGGTGTATAACATACTTTGTCACAACTCGCCGCTACGGCTCGCAT T GCAATTTGAGACTACTTTCAGTGTCTCTAAATTTATAAGAAAACTACTGTTAAAGCACAAAAATAGGAAGAAGTGAAGAACTGGTATCAATCTGTGAGATTCTG ATAGAACATAAAAAAGCTTACGATGCTTAATCCAAGAAATTCGTCTGATTACAACAATTTTT
<b>TmaSat69</b>	
<b>TmaSat70</b>	AATGCTGTATTATTTCAAATTTAAAAAATGCTTATTTGAGCCAAAAACATCACAACCTGTAAAAAGTAAATTTGAAAAATCAAGGGCTGATTCATTTTTTCAGT GTTTTTTTTTTATTTTTTTTTTTCAGATTATCCCGAAGCTGCGTAATAATTTGACATTTTCAGAGCTATTTGTTGTTCAAATAATAGTCTATTTGTTTGAAGACAA ACCTGCTTAAAAAATATCCACTTTGAGGGTAATATAATCAAAAAATTTTCCACTGTTGTTGGTGGTCTTTCAGTCTTCAAATAAATTA
<b>TmaSat71</b>	GGGCCAACTTAAAAAACAACAAAAACCAAAATTTGACCCAAAGAAAGAAAAACATCGGTTTCAAATTTCAAATTTGCTGTTTGTACTTTGAGAGTCTCCAAT ACCTCAAAAATTTCAATCAAATTTGATTGCTTATCCGCTAGTCCAAAAATGCACACTTTAGAGACTATCTTCTCAAATTTAAATACTATTTACCTCAAAATAAAGTCT ATAAAAAGTACTATTTTCAAACTGTTTTGAGTTACTTTACAGACTATTTTCTGTTTACGTTTACGGACGACTTCAGAAAAATACTTAAATCATTGTTCAAATAATCAGTT
<b>TmaSat72</b>	GCCAAAAATACAGGATTAGTTTTTAAAGGGTTAAGCAGCTTTTTAAGCATTATTTTTTGAAGCAGCAAGAAATCTATGAAAAATTCATGATGCTTCAATCCGTAATAAT TTTTTTCTGCAATTTCAAATTTGATTTTCAAAAAGATGATTTAAAGAATCGTGCAACAAATCGTCAAAAAAATAATTAATTAATTTGAAAAATGTTAAAAA GTTTTTTTTTTATTTTTTTTTTTCAGATTATCCCGTGTACTAGAAAATTTTTAATTAATAAAGAAACTTTGATAAAAAATGTGAAAAATTAATTAACGCAAAATGTT ACATAAGATTATGCTGTTGTTGCGGATTTTTTCAAATAAGTAGGATAAGGAAAGTTTTTACCATTCAAAGAAACGAATTAATTAACAAACGAATTACAATAAGATACT GGTTGCTATTAATAATATTAATAATTAACAACGCAAGTTTTGTTGATTTAGTACTCTGATTTTGAATTTTTCTGAAGTAAAAATCAAATTAACAAACGTAATGCTGTTCT GAATCACTCTTTGCTGAGGATTTCAATTTCCGAAAGACTGTCGAAAGAGCCCTACATTTCCCTACTACATGTTGGTGGGCGGAGTGTAGGCTGGGTAATG GGTGACCTTCAAATAATGATATTTTTTACCTTTTTAAGAGTGTAAAGCAGCTTTTTGAACAATTTTCGACAAAGAAATAGTTTTTATGTTTTTATGTTCAACAGAATA AGAAAAATGCAAAATTTCTCAAATAAATCAATTTTAGGTTCTTTCAAAGTGTGTTAGTATATTTTTCAAGAAAAATAGCACTATTTTGGCAATATCGTAAAAACA AACCAAAAATTTCAAAAATAAACCTAAATAGTGTAAATTTGCACTTTTATAGTTTTTCCGTTTCCAAAAATCAAAAGTATTGTTAAACTGACTGAAAAATTTACCAAT TAGATTTAAATGATAATTTTTGTTGTTTCAATTCGATTTAATACTGTTTTGAGTGAAGTTTTGCAAAAAACAAGCTAAAAACCAATAAATTTGGTGGAAAAATGTTG TACTTTATTTTTCAAGTTTTAAGCAAGTTTTGAACTAAAACACAGTTTTTTCGAGATTTTGGCAGACAGTCCACAAAACTCAATTTGCTATTTTGGCGTTT TCAGGTGTTTTAGTACGATTTTTTCAAGTAAAAAAGTAAATTTTTGCAAAATTTT
<b>TmaSat73</b>	TATGAGTCGGAAAAAGTACTACTTTACGGAATTTGAGAAAAAGTGTTTTTGTTGTTTCTCTAGTAACTGTTTAAATTAATCAATTTTCAAATAATGATGTC AAAAAACAAGTATTTTCAAAGTTGAATATAATTGATTATTGGACATTTTAAAGAAAAACAATTTAAACCTTCATAGAGAAGTTTTAAATTTGCAAAAAATTTGAAA ACCTCGCACTTTTTGGGCTATCTTGTCAATAAATGACA
<b>TmaSat74</b>	AAAAAATCATCTAAATGTTTTTAAAGTACACACTGTAGTATAAACTAAATTTTATCTTCTTCTGTTTTCGAGGGACCGTTAGTTGAAAAAATGAATAGGT ACTTCAACATAACCAAAATTTCACTGAAATTTTAGTAATTAATCTTGAATTTCAAACTAACTAAATTTCAAATAATTTCTTAAATAATTTCAAATTAAGTTCGATGTTCTAAA AAATTCACGTAATTTTGAACAAATTTTCCGATAATTTCAAATAAATTTTACAGCCGCTTAAAGAGCTCAAATCTCATGCAACACAGACTCACCTT ACATGGAAGCAAATTTACCCATTTAAAAACACACTTTTTCAAGCTAAAAATTCGCTCCGAAGATAAAACAATGGCCGTTGGGCTATAATTTACTATCTAGCAATCACT AAAACAATTAATGATAATTAACAAATCATGCAAGTTTTTAACTTTATGAAATAGACGACTTTGAAAAACACATACAGGTAACAAAAAGTGTAGAACCAGCGTAT TTTACTAACTAAAGTATTATGAAAAACGATTTGTTTCACTAATATTTTTTTTCAAATACTTTTTATTTTTTAAAGTTACCAATTTAATAACTAAACAAATTTTTTGA TCAAAGACTATGATCAACAATCAACAATAAATTTGTTTTAAATCAATAGTGTTTTTTGAATTTGTTAAAGTTGCTTTTGACATACACAAAGATTTTTTCAAT TGTACTAAAAAAGATTTTAAATGCAAGTTATCTCAAATCTTTTTGCAACTTAGGACATACAGGGTGTAACTATATATTTTT
<b>TmaSat75</b>	TGAAAAAACAATTTGAAACACAGCAAAATTAAGAATAACAAAAACAGGACACGAAACCGTTCCGCACTTTTAAATTTTTTCTTATTTTTTATTTTT CTTATTTTTGACGCACAATTTAGAAATCTGTTTTTGGAAACAAAAATTTATAGAACAACAAAAAAGTAAAAAATTAAGTATAATAATACCAGTAAATTTACAAAA CAGAAAAATTTGAAAAACAAGGACAATATAAAAAACCAAAATATAATTTTTAATAATATAAATTTGAAAAAATTTGAGAAAAATCGAAGAAAAAATCGCAAAATCAA TCTCAAAAACAACAAAAATTTATCTTAAAAATACGATCACATTTTTTATATTACAAAAA
<b>TmaSat76</b>	ATTTTTCTGTTTAAATAAACTAATTAAAAAACATGTGAGGAAACAAAAACGGGACACGAAACCGTTCCGCACTGAAATTTCTTAAATTAATTTAGGACTTTCTATT TCACTCAAATTTGAAACATGAATTAATTTGGGTCAGTGCAGGAGAGACGGAATAAGCGGGATGAGGTTGTGTGAGAGATAGACAACCTGGCCATTAATAAAA CAAGCATAATTAACAAATCATTGCAAAAAAACAACCACTACGAAAAATAAATAAATTTTGAAGAAAAATTTAAAGTGTAAAGTTTTAATAAGACATA ATAAACAAGAACATTAATAAAAAATAAGTTCTGAACTTGAACCTCGAAAGTTAGAACTGAAATCGGAAATTTTAAATTTGAAATTTGAAATTTGAAATTTGAA TCAAATTTGCTTAAACAGTCAGAAAAACAAGGATTTTTGAAACGAAAGTTTCTGAATTTAGCTTTTTCAGATCTGCTAATACATACATACTTAGCAATAATGTTTTGCT TATTATAGAAATTAACAAGTTAGAGAGACTGCAAGTTATGAGTTTTTAAATTTTTTGAAGTTAAAGTTTTG
<b>TmaSat77</b>	TAATTTTTAATCATATTTTGGCAAAATAAATAAGTCTCTATTTAAAAAACAACAACTAAATCGAGCAAAACAAGCTAAAAATAAGTCAATTTCAATATAATTTAGTAAATTT TAGACCAATACTGATTTCAATTTTTCGAAATTTTCGAGATGGCTTGGGAAAAACAAGCGAAAAAATTTGTTTCTACGAGAAAAATCAAGCACAGTGGGTTGAGGCCGG GTATGGAGGCGTGGGGACTGCACTAACTACACCAAAATACGTTGTGATAACTATAATTTTATAATTTTTTCAATTTAAATCTCGCAGATCTGGCTTAAA AAGGCAAAAATAACACATTTTGGTCTAATTTCTAATTTTTTGTACTTATGTTTAAACGAAATTTTCAAAATAGGAACGTTATACCTGAAAAACATGCTAAAAACATTT TTTTTGTTTTTATTTAGCAAAATCTGAAAAAACAAGTCTGCTTCTCACTTATAAAGACTGAAATCGGAAATTTTAAATTTGAAATTTGAAATTTGAAATTTGAA TAAATTTTGGCAAAATCTACTCAAATGAGCTAAATCAACAAAAATAACATTAATTTAGACTTATTTTTT
<b>TmaSat78</b>	AAGTATGAAAAACAAAAATAATTAATACGAATTTTTTATTTCTGGATTAATATCAAAAACAGACTTTCAGTAGAGGTAATAAATTTTAACTTTTTTAACTATTGATATT TAAACTAAATTAATTTAGAATTTATGTTTATTTCTATCCCGCACTGAAATTTTGTAGATTCTTGTGGATTGTTAGCTTAAAGTATTCTGCTCCAATGAAGTGA ATCATTATCAAAATGATTTTATTTTTCGTTATGTTGAAACACAGCAATTTTGTATTTCTTATTTAGTAAATTTTTTAACTTTTAACTTTTATGCTAAAAA TTCATTAATAATTTATGTTGTTGTTGCTTTTTCTAGAGTGCATTTTCAATTTGTTTTCGACTGACCGCAACATTAGAGAAAGAAAGTAAATAGGGAGTTGCC GTTGTCTCAACAAAGACAGATATATAGTCAAGTGAAGTATGGCAAAAGCGCGTTTTAAGCTGTTCAAACAATTTTCAATTTCTCGCTTTTTAAACATATT AAGACATTTTCTCAATTTTAAATAATTTACGTTAAGAAAGATTTTTCAATTTGATTTTACTAGGAACAGCAGGAT
<b>TmaSat79</b>	TTAGTTGACATTTTTTAAATTTTAAAGTTGCTTGAAGAAATTTGTTGAAAAATTTGTTTGTGCTTGGGCTTCTGCTCGCTACTATGCAAAATTTTGT TAGATTTTTTTTTTAAATTTCAAAGCTGTCTGATATAATTTTATGTTTGGCAGCTCGTCTGCTCGCTACTAAAGTGAATTTTATAGTTATTGACAATTT TTTTTAATTTCAATATCAAAATTTGGTAAAGTTTTGTTAATTTTATAGTTTTTGTAAATTTTTTTTCAATTTCAATATCAAAGTTGCAAAAGTTGGAAAGTTGGTATAT AAAATTTTTGTTGCGCACTCGGAGTTCACTTCGCGTACTAAAGTTAAATTT
<b>TmaSat80</b>	CGAAATCATGACGATTTTCTCGACAATTTTAAATTTGAAAAATGACCTTTTTCTGTAATAAGCAAACTAAAAATGTTTTCTAAACATAAGTGGTTACCCTGATATTT CCGTAGTTACTCTCAAGAGTAACCAAGCAAAATTTTTTCAAATAATAACAAAAATACCAAAATTTAGTTGAAAAAATGTTGTTAATGTTCTTTTTGCACTTTTTAA ACACGTTTTTTAAGATAAATTAACAACTAAAAAGACTAAAAATGATACAAAAATGACTTTAAATTTAAAAATGTTAACTAATTTGTTTTTGGCAGCTTTTTCAAG CAGAAATGCAAAATTTCTGCGAAATTTCTGCGAAATTAATAAATAACACACTTAAATTTTCTTTTTTAAACGGTTTTTATGTTTTGAAATTTGCTTAAATAATTTGCTTTCC GATGGTCTAAACATAAAGAGTTGCCACTGATATTTTCGACTTATGCTCTGAGAGTAACTAATAACAGAAATAAAAA
<b>TmaSat81</b>	ATGTAAGGCTCGAAATGCTAAAAAACAAGTACTTGTGCTGAGACTAAAAATAAATTCATATTTTCGACATTTAGAATTTTTTAAATTTGATTTGTAATTTCTAGAATT GCTAGAATCGAATTTTGAATTTCAAATAAATAAATAAAGAAAGACTAAATTTAGAAATCTAGAAATGAACGTTTTCAAATACTAAAAACACTAGTAACTTTGTTGAGA TTTTTTAAATAAAAAAGCATATTTGCACTTAGAATTTTCAAATTTTGAATTTGAGTTTTAGAAATTTCAAATCTAAGTCTAAATCCAGAGTTAACTAAAGAAAG ATTACATTAGAAATCTAGAA
<b>TmaSat82</b>	ATTACACAGTGGCTCAACATTTTAAATAACAGTCAATTTTTAATCAAATTCAGTAAAGCCATAATTTCTGTACAAATTCATTAAACAGAATAAAAAAATAAATGAA ACTGAGAAAAATGCAATTTTTCAGCTCCCGGCGCATCCACATAAATTTCAAAAACACTACAGTGTCTCAACATTTTAAAGAACTTAATCTTTTACTGAAATTTTAAAT CAAGACATAATTTTGTACAAATTTCTTACAGAAATGAAAAATAAATAAATTTCAAGTAAATTTGTTTTTAACTCGGCACATCCACAAAAATCTAACTAACTATTACA ATGTTTCAACTTTTTAATGAAACGTAATTTCTCAACTCAATTTCAATTTAAATTTAAGTTTTGAGGAAAAAATGTAATAAATTTGACTTAATCCGACAGTTCCTTA TCACAAGTGTAAATTTTCAACCTCAACGGTGTATTTCCCAACCCGATTTAAATTTTGAATTTTCTGCTAATTTAAATTTGAAATAAATTTCAACAACTATTGAAAAA
<b>TmaSat83</b>	GAGAAAAATGCTTTCTGACTGATGCAAAATAACAGAAAGTTGCGATGTTTACCCTAAAGAAAGAAATAAGACAAATAGTTGTTCTTTAATTAATCGATGGGGTAAA ATTAACAAAAACATAGGTAATAATAAGAAAGTATGAAATATATCAATGTTTTAAAAACGCAAGTTGACAAATAGTTCTGCTATTTTTGCTGTAACCTAAATTTATA TAAATTTAAGGAAAAAGTCCAAACAGCAAAATCGGCAAAATTAAGCAAAAGGATTTAGACATTTCTTATGTAATGCTGTAACCTGTAACCTTTGATGATTTGAAAA ATGAAACTTTCCGAATGTTATGGAACAGATTGATAAATTTGATAATTTGATAATTTTATTTTTCGTAACAACTAGAACTAATGAAATTAATGAAATTTGAGAG ATGAGAAATTTAAATTTCAAGAAAAATTTATTTAATTTTTTACTAATTTGAAACTATAAATTTGACGAATTTAGCAAAAAATGTTCTAACCCGCGCATCCACCAA AACTGCAATTTTTT



<b>TmaSat107</b>	GGAAGTCGGAATTCACCTCTCTGCAAACTGCAAGTGGGGAATCCCGAATTTGGTGTGAAGCTGTTGGTTATTGTCTTTGTTATAGTTTCACCTGAGGCCAAAC TGCCGGATCTTTTTCCGATTCTTAAGTGATCTCGATGAAAGTTGGCGAAATTTTGAACCTGTTTTCTTTTATTACTAAGATTTGAAGCTGTTGGTTGTCTTTGTAT ACTCCGCTGAGGCCAAACTGCCGGAGGAGAAAGTCCGAATTTGGGACTTTGAGGCTTTTTCTCTGAAAATATTCTCCGCTGGA
<b>TmaSat108</b>	ATGAATGTGTGATGTTACAAAAATGAAGATTATACAAGTCTCATCTAGCGAATACATACTACGTTTTTCTACAACGACATTTGTTTTTAAATAAAAAAAAC TTAAAGAAAGGAACTAAAACTAAAAAATTAAGAGAGACAAAAATAAAACGGGAAATGAGAGAAAGACGGCAAATGACAAATTTTAAATTTTTTACTAACTTTTT AAAGATTTTAGGCTGTTTCAATGACTTTTTAGTTATTTGTCATCTTTAAATTTTTTAGCGGATTATTAATTTTCGAGTTGTTCAITTTGATTTTTCAAAAAACAA AGACTTTTTGAGGCTTTTTCGGTATCTATAAAATGTTTTGAGGAGAAATGAGGAGTTTTTATCATTGAGACTTTATTAGATTAAAGATTGAGCCAATTGAG
<b>TmaSat109</b>	CGACCCCAATATTTACCAAGTAAAAAAAGTCAAAACATCCCTGTTTTGTATAAATCAAAAAATCCTTTCTAGATATCTCAAACGCGTTTCATTGTTGGAAC CAATAAAAAACCATAAAATTAATCCGTAATTTAGAAAATTTAAAAATTTTATTAATTCACCTGTTTTGGTTTTTTTCGTCATTTTTCGAATTAATAAGATTGTTTT ACCGACTGTAAAAATCAACAAGACGAGGAAACAAACAGTCAAGTGAAGGCAACAACGTCGATTTTTATTTCTGGACCAGGATAAATTTGTAATAATAAAAAA AA
<b>TmaSat110</b>	TTATTTAATTTTCAGAAAGTTTTTTTTGAATAAGAAATTAATCAAGCAGTATTTTTCAATTTCTGGTCTTAACTTAAATTAAGTAAAAAAATTTGGTCTTTTAAACAT TGTCACACAGATTTTTGGTATAGACTCTCAACAGCTACTGACTATTTGAAATTTGGATTTTTATGTCGTTTTTGTCCAAATGCAATAATGAGACTTTGAAATTTA AAATTCATCCCTCAGCTTTATTCGGGTCACGTGCAATTAAGTAAGTAAAAAAATAGAAAATTTCTATAACTACCAATTCATAAAAAAGTCAAAAGTCT
<b>TmaSat111</b>	TCGACATTACCAATAATCAACAATAAAATTTGCGGAGCAATGCAAAACATGTTTTCAACATGTTTTGTATCAAAAAATTCAGAAAATGCCAAAAATCTGT GATTTATAAACTAACCTCTGTTAGTCCCGATTTTGAATAAGTAAATAATAAAGTCATGCAATTTGAACCAAAAAAGCACTTTTTAATTTATGAAACGAG AAATTTGTACTAATTTGACGAAATTTCTGCAAAATTTAGCAAAATTTTTTTTTGTTTTTACAAAATAACATTTT
<b>TmaSat112</b>	CAGGTTTTACTTCTAATTTCTGGGTTTTGAACCTTCTGGAAGTGGTACGGTTTTACTCAGGTACAATGGTACTGGTCAACTTCGATTGGGATTGGTACTGTTTTAAT TCCTGTTGAACCTGCACTGGAAGAGTTAACTCCCGTTTTAATGGAGTGGGAAGGGTTTTACGCTGTACAACCTGGTACTGGTTGATTCCATTTCTGAGTA TCAACGTCAGGAGTGGTACAGGTTTTACCCAGGAACGATTGGTA
<b>TmaSat113</b>	GTAGATTTGTTCTAAATGTAAAAATCTAGTCAAAAAAGAAATTAAGAAAATAAGGAAATGAAAGAAATCTACAAAAAATTTGAAGAAAATGTAATAATTTCTAGAAAAT AGTAAACAAAAAATATAGTCTAATAGACTAAAAAAGTAAAAATAT
<b>TmaSat114</b>	TTTGAACGCTGTTTTACTAAAAATCGCTGAATAGGTAATAAAGCAAAAGCTATATCTCCATAACGCTTAAACTATCAGGAATTTTTCGGTTTTCTCGACACCAA TCGACTTCTCGGAACATTTACATAAAGTAAAGTTCAAAATAGTTCAAT
<b>TmaSat115</b>	TACAGTCTTTGAAACAAAATCTGAATTCATCTTTGATTTCCGAAAAACTTGGAAAAACCTCTTTTTATCTCAAACCTTAGAAAAAATTTGAAAAACAAAATGGT CCGCAATGGACCTTTGTGGTTAC
<b>TmaSat116</b>	TCCAAAATCTAATTTTATAAAAAACAAACAAAATAAGAAATAAATTTAAAAATAGAAAAAATATTTTTTTGTAATAATTGCAAGATTAATAAAAAATAGGCT GAAAAGCTAAAACTAAAACTCAAGAATTTAAAAATCGGATAAAAAAGCGAAAATCCAATAATCGAAGAACAAAAATCAAAACACTAAAGAGCTTCAATCCA AAAAACAAACAAAGAAATAAAGAACTTTTTTCAAAAGTAATAAATAAAAAAATAAGTATCTGAAGGTGGAAGAACTAAGGACTTGAAGAACTGAGATTTCTGAAA ACC
<b>TmaSat117</b>	TTTTATCTAAAAACAAATGCTGTACATAAAGTCTCGTGTAAATAGGAATTAATAGCCTGTTTTGAGCTGAAAAACGGTCCGATTGACAGTTGGGCACTTTGTCTATT ATCGCAAAACACCTGAAAAATACTGAAAAACAGTTGCGTGGATGTCCTGCGGGG
<b>TmaSat118</b>	AAAATGTAATTTCTAAATTAATAAACAGAAAGTAAACAAATAAAAGCAAAACGTAACCTGTTAAAAATTTTATGGTAGTTAAAAATTAAGGTTTTGTGCT TGTTACACAAAAATTAAGGTTGCATAAAGCTATTTTCGAAATATCGACGCTTTTTGCTCAATAATACCGAAAAATGAAAGCTTAAAGCTACTGAAAACTAGAAAA ACAAAGTCAAAAGCTCGTTTTGGGAGTTGAAAACACAAACAGCCCAAAAAAATTTCTTCAAAGATTTAAACGTTGTAATAAAATTCACAACCTTCAAAATTTA TACTTAA
<b>TmaSat119</b>	TTGGCGATAAAATTTGATTCACCTGTATAAACAGAAATCGTATATCAACATTAATAAAATTTCTAGTCTAATTTCAACATATTTTTTATACAAAACTTATTAAT AAATTTAATAAATTTAGAAATAAACTTGATTCCTCTGTATAATTTGTAAAAAATCTCCGCAAAATTTATTTATTTCTTTATTTAAG
<b>TmaSat120</b>	TAGTGCATTTCAAGTTAGTAGGCTGTAAAAAATAAAACAAAGATCTTAAATTCGTAATCTCTGTTGGGCAAGCAAGAAAAATGCATAATTAGCGTCAAATG ACGTAATTTGTTTTCTCCGTTGGCCAGAAATAAAAAAATACTATATTTTTATAGTAGGCAATGAAAAAGTTTTA
<b>TmaSat121</b>	ACGAGTTGAAAAATTAATTCGTTACACTCTATATAAAATATTTCTAAAAATGAAATGTTTTGAAAGAGCGTTTTCAAGTATAAAAAAGACCCAAAAACACTG AGTAAAAACATTACGAAACAAGTATCTACTTTGCTTTTTTAGCTCTGTATATTTTTTAATTAATCAATACAGTTTTTCAATAAAATTCATTAAGAAAAGAGTAAACCT AGTTTTGGGAAAGTAAATTTGGAGACATATTGGAAAA
<b>TmaSat122</b>	CAGTTGCAATGTTGCCATTTCTAGATATTTTTGATTATTTTTTAGTATTGACTCATTTTTCTTCTTAATTTGTCAAAACATTCAGCTGTGACCTTTGTTTTAAGATC GATTTGACGTAATAAAAAA
<b>TmaSat123</b>	GATTTTTCTAATAAAAGTGTTCATCTCAAAATTTCTATTCTTCTATTTTTTAAGATTTAACATCTCAAAGTTTTACCACCTTTAATTTCAAGATTTAA ACTACATTTTCAAAATTTGAATTTAAAAAATTTGTTTCACTCACAATCATATTTTTCTTGCACAAAAGAGATTTATCACCGTTTTCAAAATTTTTTCAAAATTCATT TTTTTAGTTAACTCTAACATTTTTCAAAATCTCTACTTTAACATTTCTCAAAATTTAAAAATTTCTCTGTTTTGAAATTTCTAGTCAATTAAGTATTTTTTA GTTTTTGGACTCTCTAAGCTGTTGAAATTTTTTATTTGAAATCTCAGATTTTTAACCACTCTTAAATGAAATCTTGAACCTTATCAAATGACGTTTTTTAATTTCTCA
<b>TmaSat124</b>	CTTTTTTTAACTTTGACTAACTTTCTGTTTAGAAGCTGCTGACGATTGCGAGAAATAGGACCCTGACACTTTTTT
<b>TcoSat01</b>	AGCTGAGATAAATTTGCAAAATTTTGTTCAGAGTGATAGAAAATCAATATATAAATTTGCAACAGAGCCATAAAATGAAAAATAAATTTATGATACGTAGTTCTGGA GACCTAGCTATAACACAGTAAAAATTTTATGCTTTACTCTACTAAT
<b>TcoSat02</b>	TCCTACTTTTATAACGTTTCAGCAATGTAATAAAAAAATAAAAAAATACGCAAGTTTAAAGATAGTCTGTTATTCTTGTATAAATACCAATTTCAAATTCATTCT GAGCTGCTAATTAATTTGAGAAAGTTGAAACAAATACCAATTCGATTGAGCAGAAAAATTTCTATCAAAATACA
<b>TcoSat03</b>	CCTCAGTTTTATAACATTTAAATAACGTTGTATATAGAAATCAAAAGAAATGCAAAAGTATAAAAAAATCAATAGTACTCCCTGTTAAAAATCAAAATCAATCTACATTT CAGGCTTTTAAATGGTGAATAATGAAACAAATCTATTGCTTACGATTCAGCAAAATTTTCTATCAATAACA
<b>TcoSat04</b>	TTTAAATACATGTAACAATCTTAGTTATAAACGTTTACTATCAGGCTTAAAAATGACAAAAACTCATAAAATGCGTAAAAATTTGGTAGTCAGCTATCAATTTGGAAAT TGAAAAATGATTTTTAAAAACTGTAGCTGACATATTTCTCTTATGCTCAAAATGTCCAAATCTCAATCTC
<b>TcoSat05</b>	CAATATGGAGGAATAAAATATAAATTTATTACTGAATTAACATGAGTTTACAATAAATTTGGTACAAGTTTCAATCTGTAGCTGATAAAGTTTCGAGATAATTATA TGTTTTAAATTTCAAAAT
<b>TcoSat06</b>	GGAACGAAATCTGAAGCAGGATTAATACAGAATGTTGTTCTGACCTGCTATCTAAATACCCAGTCGTTAATTTGAGATTGAAAGTGAAGAAACGAAATTAGT GAAGAAATCCACGGTGATTTAGCCACACGCT
<b>TcoSat07</b>	CCAGTCTTCACTGAATCATCTCTCAAGTGAAGAAACGACGCTGACGAGTTTACTCCAACCCAGTCCTGACTGAATCTACAAGTGAATAACAACGTCACGCGAA TTACTCCAAC
<b>TcoSat08</b>	CCATTATTTAAAAAATAATTTGCTCCCTCGAATCATTTTTCTGGGAAATAACCTCTACTCTGTAAACAAAAATCGCTGAAAAATTTAATAATGGAATTTCTGGTT AAGATGTTCTTTCCCTCTGACTTACAAATTTTATTCGAGATTTTAAATCCATTTATATTTAACTAATTTTACAAAAAATGACAAAAAATTTATTTATTTATGTA TTATTTATTTTACACTTTATAGCATAGTTGCCGAAGAACATTTGCTAACTCGGAGTATGCTACTAGCGGATTTAGTTTAAATTTGATTTGATGCTACGAGC TTTTTACTACTACTGAGGTTTAAATTTATTTATAAATTTGAATAAATGTTTTAAAAATGTAACAATAAATAAACAATGCGGAATTAATCTGGTAAATACGGT TTATTTCTGTTTCTGATTTACTATTTTCGATTTACCTTTTACAACAAATTTTATCAACGTTGTGAAATGGTATTTTCAACTAACCATACAAGTAACTCT
<b>TcoSat09</b>	GAAAAGGCACCTACTCTAAAGACAAAATCCCTCTCTGAGAAACCTACTGAAATTAATTTGACACCTCAAGAAATTAAGGAGAAGGATAAGTACCAGAGAAAGAA TTAGCGAAAGAC
<b>TcoSat10</b>	TGTAGAAAAATTTCAAGTATAAATGAAAGAAAAATAATTTTTTACGAAGTACAGCGTTTTGAGTAAGAAGTGCAGCGTTTTGGTTTTAACTAAGAAGTACGGCGAT TGAAATTTAAAAATTTATTTTAGAGTCCAAATGCAAACTAAAGCATTTGTATATACAAGAAAGTGA
<b>TcoSat11</b>	ACTGACCGCGCCGACGTTGCTTAACTTCGTTGATCAGACGAGAACCCTGCTTCAACGTTGATGGTCTGTTGCGTATGCTATGATATTTGAAATTAATAACAA ATACCTTTAACGAGTTTGGCTTATCTTATCTGCAATAGGTATAGTTTATGACAGTTTTGACGAACAGCAGAGTAGTTATCTGTACGCTTTGCAAAATTTCACTGTAT TAGACGGATTAACGATCATTGACTACGCTCCAGTTACACAAAATCTTAATTTTGGAAATCTCACTATCTTTTATGTTGCTTATCATTATCTCGCAATTTGGTTAATG ATTGCAAAATTCAGCAACAGCAGTGAATTTTACTATTCTGTACTTAGTTTTTTCGAAATTCGACAGTATTAACGAGGAACTATCAACGATCTTTCAGCTCT CGGTCAGTTAAACAAAAATTTCTGCGGTAACGGGCATACGCAACCAATTTTCAAAAAATTTATCTTTACGTTGTTAATTTTCTATTGCGGCTTCCCTGTTTATAAAT TCGAAATAACTTTTTTTTTCAGTTTATAATCAAATTCAGGAAAAACGCAAAAGGCAACGGCACGCGGTTCCCAAGCGGTCACCATCAAGTACT

<b>TcoSat12</b>	TTAAAAATTTTATACATTGGGAAAAATCTGAAATAATTCATGGTGGTACTACATAACATCTAATAACGTTTTTATAAAAAATTTCTTGCATTTAAATGGGGCTGC AAAAATGGGGGTACAAAAATGGTAAATTTACGTATGTAAGTTTAAAAATTTATTTGACCCCAAAATATTAATTTGAAGAAAAAGAGACTATTAAATTTCCGGGCTTG TTTTTAATTTTACAAATTTGATAAAATCTTTCTTCTGAAATGGGGCTTAGGGCTCCACATCGTTGACAAATAAACCTTCATGGCCCAATTAACCGGAGTGTCTGAGAAAAAG ATTTAAAAATATTGGACACTATTAAAAAATCAGCCATTTATATCTCCAAGTTTCAAAATTTTTGAACCATAAAAGAAAGTTGTCTACAACCCTGTACGAATACC AAGTTTGAGCTCTGTTAATGGAAACAAAAATGTCGGATTTTTAAAAATTTTTTTTTTAGATCGATAGATATTAACGTGGAATGAATGGGAAAAATATTGGTTGTGAGC CCATTTACTCTGTAGCCCCAGACGCGCTTAAATTTAAAAAATAAAAA
<b>TcoSat13</b>	ATTGCGCAATTTTACAACTGTATTGTCAAAACTACGAGAGTTGTGCAACCATTTCTGTCTTAAATTGTCAAAATTTCTGTTCTTTGACAATAAATAAACCAATTTT GTTGTAAACTAGTGATTTATAGTTAAAAATGACAAAACCTCAAATTAACAAGAATTTGTGGTTTTTGAA
<b>TcoSat14</b>	TCTAGAATCTATGCATTCATTTTTGATAACATCTCTCATCGTAGATTTTCTGTTCTTTACTGGTTGACTCAGAAAAACACCTTGT
<b>TcoSat15</b>	AACATTTAGAAATTTCTATGAGTGTGTTGCGATTCTAGAACAATCTAGATTATTTGCTTTAATTTAAATAATGTTTTAAACATTTAGAACATTTCTATAGAGTGAT GATAAAGAAATACACATTTTTAATTTTGT
<b>TcoSat16</b>	AACATTTAGAAATTTCTAGACGAGTATTATCGATTCTAGAACAATCTAGATTATTTGCTTTAATTTCAAAAGATTTTAAACATTTAGAACATTTAAAAATTTTGA ACGGAGTTCTA
<b>TcoSat17</b>	AACATTTAGAAATTTCTGATAAAATGACGCTCTAGAATTTTCTGAAATTTATTAGAAATGCTCTAGACCTTCTAGAATATTTCTGTGTTCTAGAA TGTCGAATTTACGTAATTTCTGAACATTTCTAGAATATTTTATAGAAGAATTTAGACAAATTTCTATTTCTATGTAATGTTTCAACATTTCTAG
<b>TcoSat18</b>	AAGCTTCTAGAATTTCTATAATATATTTATGTCATCTTAAACATTTCTAGAATATTTTCTGAGCCGATTAAGAATTTACGTTTACACTAAACATTTCTAGAATTTCTA TAAAAGGAAAAACAGAGAATGTAACATTTTCTGTTAGAATTTG
<b>TcoSat19</b>	AACATTTAGAACATTTCTATATAGATGATATTAACAAATTTTATATTTTCTGATAAATGAGTCTTCTAGAATTTCTGAAATAAATTTGGGTTGTCTTAAACATTTCTAG AATATTTCTATTTCTACTGTCAAATTTACGTAATTTTA
<b>TcoSat20</b>	AACATTTAGAAATTTTCCCGCAGCGCACTATCAAACTACGTTATCATAAAACATTTAGAAATTTCTGTTAATAAAAAATGATGTTTTAAGCCTTCTAGAATTTCTA GAGGTGATAACAAATCAATTAATATATCTGTTAAATTAGAGTTTCTCAGATCTTCAAAATATATTTACATTTTATA
<b>TcoSat21</b>	AACATTTAGAAATTTGAAATGTAAGAATTTCTAATCTAGAACATTTCTAGATTATTTGTTTTATTGTCCTAATAGTTTGAACATTTCTAGAATTTCTATAAAGTGACA GTAATTTAAAGCTTCTGATTTCTGTAATATTTACGTTAATTTAAACATTTCTAGAATTTCTAAGAGACAAACTCAAACTGAGAACATTTCTAGATTATTTTCTA AAACATATTTACGTTTCTAACAATTTCTAGGCAATTTCTGAAATTTTCTTACCAGATTTCAAAATTTGATGTTACTTA
<b>TcoSat22</b>	AACATTTAGAAATTTTGTGATAAAAATCTGATTCTAGAACAATTTAGAGGCTTTGATTTATTTCTAGAACATTTCTAATTTTCTAGAATATTTTAAACATACATCTA GAACAACCTA
<b>TcoSat23</b>	AACATTTAGAAATTTTCAAAATCACTATCAATTTCTAGAACAATTTAAATTAATTTGCTTTATTTATAGATTGTAACAATCTAGAACATTTCTATAAAGAAATAG TAAAACATTTTGTATTTTTTCAAAATGAAAGCTTCTAGAATTTTAAAAATTTATTACGCTGTTCTA
<b>TcoSat24</b>	ATGTTGGCAACATAACTAGGACATTTACGTTATTTTCAAGTAAAAAATTAACCTGATTGGATTTTTTATGAGGATTGAATAGTAATAATAGACCTAATCCGACATAAAAAAC AAAACAAATTTCAAGTTATTGAATTTTTTGGAGACGCTGCTCGCTTTTATTTTAAAAAATTTACCATAATGTTATAACAGT
<b>TcoSat25</b>	CTTTATAAGAATGATTGACACCCGAATTTGCTTTTAAACCTCCAGTGAGAGACATAGATATTAACAACATATTAATAAAAAAATTTATTGATTGATAAAT AGTATATAAAA
<b>TcoSat26</b>	TGTAGATGTTGGCGAAGTGTGGTGAAGTTGTAGATTCTGTAGTCTGATGTTGGCGAAGAAGTGTATTTCTGTTGTTGATGTTGTAGAATCAGTAGT
<b>TcoSat27</b>	ATTGTCGTTTTGAAAGTTTTTCAAAACATTTCTGTTCTTTTCTGAAATTTCTTTTTTATTCTTTTTTCTAAACGTAGTTTTCTGTTCTGTCTATTTCAATTTGCAATTT GATGTTATTTGCAACTTTCTTTTTCTTTCTCCGAAATTTCTTTCTCTTTGTTCTAAACGTAGTTTTCTGTTCTTTTTCTTGAATTTGTTGTTTTGAAATTTATTTCT GGAACATTTTGTCTATTTAGAATTTGTTTTTACTAAACGTTGTTTTCTGTTTTTTTTTTCTTTCA
<b>TcoSat28</b>	TCCGTTTTTATAGACTAAAAATGACGACAAAAATTTGATTTAAACAATATCGGTAGCACATAAATAGCACGTCATTTGCTACTACATTTGAGCTGAAAAAACAATTTT CCGAAAAATCGAGTCATATCGAGCCAGACCCGCACTTTTCAACAAAAA
<b>TcoSat29</b>	GCATGACCCCTGCGCAAGGATAAAAGAAACAATTTCAAAAGACAACTGTTTTAAAAATTTACCATATTTACTAAAAATTTACAGAAAAAGTAGCATGACA
<b>TcoSat30</b>	GAATTTAAAAAGCTTTAATACATTTTTTTCATCAATTTATTTCAAGGGGCAAGCCGTGATGATCAGGCAACATGTCGCTTTTACCCTCCCTTTACTTACAG TTTTGTTGGCCTTCAGTCAATTTTTACGCTTTTACGCGCCATAAATGTTTTTTTTGATTAGACATTTAAACCTGCGTGAATAGACAAATTTTCAAGTTTTTTTCAAC TAAAAAACAGTTTGTATTTTTAAATTTTGAAGTTAAGAATTTCTCAAGTGTGGCTGCAAGCAAGGGTTTTATTTAGTTAAAAATACTTACCAATTTTCTATTTATTT GTATTGTTTTTATAAATCATTAAAAACATTTATAAACCACTAAAAGAGAGAAAAATTTTTGGTGGCTGAAAAATTTTTGTTGTTGATTGTTGCGCCGTAATCACA ACGACAACGATTTAAAAATAAATTAATTTTTTACTGGAAGACAGCTCCGATTTAAAAAGTCTTCTATATTCAAGGGGGCAAGTTCTATATAAATCAGAGTCAAC ATGTCGATCTTTGACGCTCTTTTACATACATTTTCAAGAAATTTAGTTTTTGTGTTGCTGCAAAAGATGAATTTGTTCAATTTAAAAATTTTCAAAATTTGTTCTATTA ATTGATGTTGTTAGATT
<b>TcoSat31</b>	ACTCACTACAAAAAACAAGTCCACACGAAAGAATATTAAGTTTTAAAAACATTTATTTAAAGAAATAAGAAATATTGCTGACAATTTTCTAAAAATAATAGCTGGT TTTACTGCTAGTTTTAGTTATCACTTGAACAAAACTTAAACAAATTTACTAGACAAATTTAAACAAAAATATGTCGAAGCTGTTGCGAGCAAAATTTACTGGAACAACA GCTTAACTTCCGTTAAATTTTCAAAATTTAAACCTGTTCCCAAGTA
<b>TcoSat32</b>	CTGTTTTATAACTCAAAATTTTAAAAATTTGAGAAATTTCAAAAAATTTCAAATATTTTGGTCATAAAAAATAGAAATTTCAAAATTTTTTAAACGCAATTTGTACAAGACGT TTCTCTTAAATTTACGTAATTAATTTATATCGGGTGTGCAAAAAATCCCGTTTTTTG
<b>TcoSat33</b>	GAAATACAACAAAATTTGAGGAATTTGTCTCAATTTAAATGAGAAACAGCTAATAACTGCTCAGTAAAAATTTGAAAAATTTGGTGGATGCGCCGGTTATAAAAAAC TAAAAAATTTTACGACTTTTATTAACAATAAAGACAGTAAAAATCTTAAATTTGTTTATAACAATTTGATTTTGTGTA
<b>TcoSat34</b>	CAACAACATCAGTTTTAAAAATAAATTTGATTTATATTTGAATTTGTTGTCACAAGATATTACTGTAAAAACCAAAATCTTTTTGAAAAATAGGTATATGTTGA AAGTTCCCTTTTCAAAAAAATTTACTGTTTGAACCTCGTAT
<b>TcoSat35</b>	GAAATACCTCTCGCACTCTATGATCCTGATACAAAAATGAAAAATCACAATTTTTTAAA
<b>TcoSat36</b>	TTTGAATAATCTCTCGCATTTCTGTGATCCTGGTACAAAAATGAAAAATTCAGAAAAATTTACAATTTAACAATAAATGGAATTTTTCAATTTGTATCAGGATCACAG AAATGCGAGGAGATTTGA
<b>TcoSat37</b>	TAAAGACCTCTCGCACATTTCTATGATCCTGATACAGAATCGAGTATTATTGAAATTTTGAGAA
<b>TcoSat38</b>	ACAAGAACCAAGCAGTGGCAAAGTAGACTAAATCTAAAAAATAAGTTTTTTTTCGATTTAATGTCGTCAAAAATAGTAAAAAGAGTGGTCTGGTATCTGAAA ACTTGCAGAAAAATCTCCAAAAACCGAAACCTGAGAAAGCAAAAAAGAGGAGTAAAAAGAAACGTTTTTACATCTTCAATTTACAGACTCTGAGTAAAAATATATAAAT ATTGCAATTAAGAACACGCAATGAGCACTACGGAAGTTTTTTTCAACGATATGCTCCACCGTATTGCTGTAGAGGCAAAAAATTTGACACGGTACAGCAAGAGTTG ACCATGACTAGTTAGAAGGTCTAGAACAGCAAAAAATTTATGTAAGTGAAGGAGTGGCCAGTCATGCTTTTTCT
<b>TcoSat39</b>	TGTGACAGAGACGGAGAACTGTCTGAGATTGATTCAGACAGTTTAACTGATAAAAAATAATTTGAGAGAGATAGAAATAGCAGAAACAGAAATTTGTTG GAAAAAATTTGCTTAGATTGTTTATACACGAAATTTGAAACAGGTTTGAACCAACGAGATTTGATTTGATTCTACTGCAATATATTAATTTAAAAA
<b>TcoSat40</b>	TAGTCTTTATCCAATTTAAGAATTTAACAAGAGTCTTGAATTTATGTTCTATTCGCTCTATCTTAACTGCTTAAATCTATAAGAATTTAATCTGAAATTTCT CACTTATTAATTTGTTTGAACAAATGCTTCTGCTTTATAAGATTTTGAAGAT
<b>TcoSat41</b>	TTAGCTAAAGTCACTAAAAAGAGAGAAATTTGAAAACGGTTTTCTGTTAATTTAGAAATTTACAGAACGAATTTGCAAGTTTTAAATTTCCCGCAAAATACGAA TTGACGTTAAAAAGACAGTAATGATTGCCAACATACAAAAAA
<b>TcoSat42</b>	CTATGGTGTTTACCTTTGCAAAAGCAAAAGATGCAAAACGATTAAGGCTCTCACTTACTAATTTGACCTGATCCTATAAAAAATAAGTTTTGAAATAAAAATAATGCTA CTCCATGAGTTTTTACTCGTGTGCAAGCTTATCTTTCTCATTTTTTATTGATATTTGGATGTTGTTAATATCTATGCTTTTTTGAATATGTTGCTTGGTCA AGTAAAGTTTTGCTTACGTTACGTTGCGCGTTTTTAAATAATTTTCT
<b>TcoSat43</b>	GATTTAAATCTAGTCATGTAGACAAGATTAATTTGGCTAGTTGCGGTTATAGTAATAAGACTAGATGTTTACTAGGCTACACTAGTTTAAATAGGCATATATAAAA AATAAGAAAACTAAGTATTACTTTTTGAAATTTCTTTGTAACCTTATTTAAGCACTGCAATTTGTAAGAAACCTAGTCACATTAAGCTAGATTAGTCAATTAGATGCT TTTGAGTAATTTGCGACAAAAACTATACTTAATTTAGCTTAGTTATAACAATCAATAAATTAGGCTAACCAAGATAGATAGACTAATTTCTGTTGAAATTTTATAA





<b>TcoSat72</b>	TCAAATTTAGTTAACTGCTCTAAAATAAACAACTTAAGACGTATAAACACAGTAAAGTAACCTTTAATCGTTAAAATAACGTAACGTTTCGCAACACTTTAACGTACTG TAATCCGGTAATAAATCAAGCTTTTAACTAAGTGTGCTACTGCTCTT
<b>TcoSat73</b>	CCTCAAAATCAAACAAAATTTATTTGAATTTAAAGCCCTTTTGAACCTTTTATGTAATAGAACTGCTTAATATACAAGATTCTTAAAGCATCCCATTGGGACAATTT AACAGATTTTAGCTTTTATTTAATTAAGTGTCTATTATTGATGGCCTTTTAAATGTCTTTCTGTC
<b>TcoSat74</b>	TCATCAAAAATAAATCAAACAGTCATTTGTAGTGAGATTTATAGAGTCATTTCAATTTAAA
<b>TcoSat75</b>	CGGAATAAAGGTTTCTTAGGTAACAAAGAGAAGAGAAAAGGATTTCGTAAGTACAGAAGGTTATTGTAAGAGAAATATGAGACTTGGAAAGAAAAGAAACGTA ATGAAGACCTAGCAAAGTACAAATGTTGGGAAAGAAAGTAAACAGCAAATTTAGACGAAATTTAAAGAAAAAAGAAAGAGAAAGTTCTTGGGAAAACAAAAA ATGAAGAAGAAAATCTGAGGGTACAAGAGGTAATCTAAAAGAAATGATAAGGTTTGGAAAATTAAGAGTAGCAAATAAAGATTCTAGAAGAGAAAAAGA TTCAAGAAGAGCTTGGTAAATGGCGTTAAGACGTAATGAAGACCTAGCGAAATGACAATTTGAAGGAAAGAAAGTAAACAAATTTCCAGAAAGAAATTTAAAGACAT GAAGAAAATTGA
<b>TcoSat76</b>	AGTCAATTAAGCTGAGTCAACATGTTTTAACTGTTTTAGTGAAGACAAGACTCAGATTGTATGAATCAATCAAGAACTGTTTTGATTGGACACACAATATAGC CAATTTGAAATACCAATTTCTAGCATTGCAAGTAATCAATTAATGTGGAACCTTTATTTATAAAAATTTAAAAAATAACAAAAATCAGATGAATTTAAGTGT CAGTCGAGTCATTTAATAATTG
<b>TcoSat77</b>	CATTAGACAGTAATATACACACTTAATATATTTTCGGCGGTCTGTTTTTAGTTCATTTAAACACTGATAATTTGACTTTTTAATTTATCTTTTCGACATGATAT CTTTTAGATCATTTAGACATTTGATATATACCTTTTAAATTTTCTCGACGGCCATGTTTTTAGATCAATTTGGACACTAATATACAAATTTTCTCGAGGGTCATAT TTTTTAGAT
<b>TcoSat78</b>	TAATTTGGGATTAACAATGTTTTCAATTTTTTAAATGAGTCAATACAGGATTAATAAATTAACAGTCATAAACGACATTTGGAATTAATAATCTGTTTTTGAATTAC ACAGTTGGGATTAATAAATTTTGAACAACTATTTTCTAATCCGG
<b>TcoSat79</b>	TTATTACAGTAAAACAGCTCTATTGTTACATAATTTAATTTTTTTTGTAAATTTACAGTTATAGCCAGCCGTAATGACTTTGTTTTGTAAGACGCTACATTCAGAGAC AAAAAAAATCGTTTTAACTGTTTTCACTGTATTTGTGTTACATTTTAACTCAGCTCTATC
<b>TcoSat80</b>	CTAATTTCAAATTTACTAATTTACAAAAAATGTTCTGTTTTAGATATTAAGCTTGGAGACCCATTTACTAATTTAAAAAATTTAATTGCAATTTACTAATTTATAA ATTAGAGATTTATTTACTAATTTTGAAGCTAAAAAATGTTTTTTA
<b>TcoSat81</b>	CTAATTTTAAAGTAATAAGAAAACTTTTTTTTTCATGAGAAGCTTGGAGAAGTCAATTTACTAATTTGAGAATGTTAATTTACTAATTTTAAAAAG CAATCTCACTAATTTTGAAGAACTAATAATGTTTTTACTAATTTTTTGTACTAATAAATAAAGCATGTTTTGACATTAGAGGCTGGAAGCGTTTTTACTA ATTTGAGAATTTCTAATTTGTAATTTTACTAATTTTAAAGGAAATATTTTAGTAATTTGAAAACCAAAACCAATGCTTTTCTAATTTTAGCTTAATAAATAAAAAAC ATTTTTTATATAAAAAGTTGAGAGCTCATTCTAATTTGACTTATTTAAAAACAATTTTACTAATTTTGGCAGTCAAAAACCATGATTTTTA
<b>TcoSat82</b>	AATTTTCAAATTTAGTAATGACAAAAAGCAGCTTTCTTTCTATTCTGAAAATGCAAGACAATTTGAAAAGTCCGAGACAATTTTACTAATTTGAAAACATAGATATA TGCTTACGTAATTTAGAAGTCAAAAGCGCGCTTTCTAATA
<b>TcoSat83</b>	CTAAGAGTTAGAAGAGATATTTCACTAATTTAAGAGCTAGAAGGATGATTTCACTGATTTAAGAAGCAAGAAGTGCATTTCACTAATTTCAAGCTAGATCCAACA TTTTACTAGTTTTAGAAGCTAGAATATACATTTCACTAATTTAAGAAGCTAAAAAGGGACATTTCACTAATTTTCAAGCAAGAAGGGACATTTCACTAAT
<b>TcoSat84</b>	CTAATTTTAAATGTAATAAAGCGGCAATTTTCTAATTTTAAATCGAAAAACCAATTTTGTAGTTTTGAAAACCGCATGTGACATTTTACTAATTTTATA AGCTAAAAACAATTTTTTTA
<b>TcoSat85</b>	CTAATTTTAAATGTAATAAAGCGGCAATTTGACACTTTTTATAATTTTGAAGAACTGCCAAGCAGAAATTTACTAATTTTGAAGCTAAAAATCGATTTTACTAA TTTTGAAATACAGCAATGTAATTTTACAATTTTAAACCCGCTAGAAACATTTTACTAATTTTGAAGAGCTAGAATAGACATTTTATTGTTTTGTAACCATCAGAA GCTTAAAAACGACATTTTTTATTTTCTAATTTAAGAAATGACATTTTACTAATTTTAAATACAGAAATGTAATTTA
<b>TcoSat86</b>	GTAATCACATTTAACTCTTCTACTGTTGTTATTGAGTGTTTAAATCCACATTTAACTGTTTTAAACAGTATTTATCAGATGAGTTTTACAAGTTTTGTTGCTCAT TTTTCAAGCTTGAATTCGTCTACAAAATTTTTTTT
<b>TcoSat87</b>	ATTAATTTGATTTACCGACATATTGACGCATCTTTAGCAACAATTTAGAGTAGAATTTGATTTCTAAAAGAGTTTTGAAAGCACGCAATTTGCAAGAACTATGA TTATATTTACTAATAAAGAAATTTGGAGTTATTAATTTTAAATTAATTAACGATTTTCTA
<b>TcoSat88</b>	TAGGTGCAAAACGGACCAATCTTTGTAAAAGTGCCTTAAATTTGACCAAAATTTGAAAAGTACCCTAAATAGTACTCGGAACTGAGATTAAGAAACCCA ATTTTGGTAAACAGTACCTTAATTTTGGAAAGCGAACCTAAACATTTGAAAAGTGCCTTAAATATATACCGAAGCTTAGATTTGCTAATAGTAACTTATAAATGT GGTCTAGAAAGCCAACTCAACATCTATTAAGCAATGCTTTA
<b>TcoSat89</b>	ACCAATTCACAATTTCTATTTCACTAACCTAGAACTTCCCAATTTGCTATTCTATAGCTTTACTTTTATAGTTTTATGACTCTATAAATCTATAATTTGACTTTTTACT TTGTAAGAAATTTAGAAATTTCACTATAATAAATTTCAACAATTTTGAACAATTTTACTGTTTTTACCATACCAGAAATCGATTTTCACTGTTTTACAATATATACGT TGGCTGTTTACAGAGCTAGAATTTTGTAGTTCTTATATAAATCAGAAATTTCTGTTTTTCTGTTTTCAACATTTTCAACTTTGGCAGTTCTACTGTTCT
<b>TcoSat90</b>	TAAGACAGTTAAGAAGCTAAGAGCATTGCAAGAGGTTGCTTTGACTTAAAAATGTTGTTTTTAAAGCTTCTGGAGTTGATAGAACAATTTTTCGCGGCTGT TGTTTTCTCAGAAGATTTGAAATGAAACAAAGACTACTCTTAAAGAACTTATAAAT
<b>TcoSat91</b>	GTTACTGTCTGTATAAACCGTTTTAATTTTAGTGAAGCAATAAATTTAGAACTAGAATATTGCTGGAAAATCAAATTTGAATGCTTATACTGTTTTCTAAAG TTAGAATTTGCTTAAAAATTTATATAAATGTTATAGATTTTTTGTGATAAAAAAT
<b>TcoSat92</b>	TTAATAACTTAAAAACACTACTTTAAATAGCTTAAAAACACTGCTTTAAAAGTAAAAAGACTGCTTCTATTGGATTAAAAAGACTGCTTTAATAGCTTAAAAACGCT GCTTTAATAGCTTAAAAACACTGCT
<b>TcoSat93</b>	TTATTAGATTTCTGATATATTTCAAGTGGTTTTAGTAATAAACAGAGCAATTTAGCGGTTCTAGATGTACATTTTGAAGACTTTAAACAATTTACTATTTTCTGTA ACTAATAAATATATTTTAGTATACGTGTTTTCAAGAAATTT
<b>TcoSat94</b>	TTCAACAACTCTAATAATTTACCCTTCTGATACCTAATATACTAGAGTGTCTTAAATCAGAATCAGAAGATCTAGATTAACAGCTTCTATTAATCTAGAATTT CTGCTTATAATAGTAATTGAGCAGAAATTAAGACTTTCTCTATTTTATTTAATTTGGTATTTCTGAATCAGAAGC
<b>TcoSat95</b>	CAAGAAACAATTTAGAAGATTTCAAAAATTTTGTAGAAGAAGAAGAAAAACAAGAAACAATTTGAAGAAACTGATAAATAGTGTGTTGAAGAGAAAGA ATTTGAGAAAGAATAATTAATAAAGAAAGAACTGTAGATAAAGCAATTTGAAGAAAAAGAAAGTACAGAAAAATTTAGAAGAAATGCAATTTAATAGTATTA GAAGAAAAAGAAACAGAAGAACGTAACCGACTTAAAAACAGTAAAAA
<b>TcoSat96</b>	AGCTGTCAATTTTACTACTCTTTGTTACTAGTGCATATACAATAGTTTAAATACAAAATAAATGATTTAATGATTTATTGTGAC
<b>TcoSat97</b>	TTAATATACAAGATGGCTAATTAAGAAATTTAATTTCTCATTTAAAAAATTTGTTGGATTGTTGATGGCCCTTTAGAGACCCCTTCTAGCCCCAGAGGGGCCCT TTTTATCTCCCCAACTGCTTAATATCAAAAATTTTCAACAAATCTATTGTGCAATTAATTTAATTTTCTAATTTTAAATTTTGTCTAATTTTGTCTGAGTATAATTT CTATCGCCCGGAAAAGGGTCCATTTTATTTTCTAAGTGTATTAATATATCAAAATTTATGACCAATTTTATGACACAATTTAAACAAATACAGTTTTTCACTTTTAT ATTTTGTGTAATTTGAGTGGGATCCCTTCTATTGCTTAAAAGGGGTCACCACTTCTGGCCAATTTATTGTC
<b>TcoSat98</b>	GATAAGAAAACACTAGTGTCTTTCAACCTCTCAACATCTAATAATGAAAATTTTTAAAAACAACCACTTTCAGAAATTTCAACATCACCATCATCTGAAAAAT AATGTTACAATAACTTCAAGTACGGATAGTAAAAATGAAATTCAGAAAACTCCGAGGCACTTCAAACTAATAGGTAATTTACAATTTCACTAATGAAGT ATATGTGTCGACCAAGCTTCAAAAACAGAACTCGAAACAACCTCAAAAACAACCTTCAACCAACAGACGGTGTAAAAATAACTACTAATTTACGACTGAAAGAAA TTTTTCAAAAAAGAA
<b>TcoSat99</b>	CGGCCACAATTTGTCACAACAATTTTCAAAAATACGCGACTCAGGTTTTAATTAAGAGAAATCTTTGATTTTTCAGTTATTGCGTTGGTATTCTGCGAATTTCTTA GTCTGATTTTTGACTGTGTTGTTGGAGTAGCACATGTGCAATTTTAAAGCCGTGAGGCACTCAGAC
<b>TcoSat100</b>	TGGTCTTTTTATGGGTATCTCCCATCTGGCAAAATTTGGTGTTTTTTTCGCGCTGCCCT
<b>TcoSat101</b>	AAAGATTAGCATGGCCCTGCGCAAGAATAAAGGCAAAATTTTCTCACTTAGAATGATTTGGACCTGACCTGCTTGAATTAATCTGCAATACTA
<b>TcoSat102</b>	CTTCTTTGCAAAATCTGCTTAAACATGTTAATGTTGATAACAGGGCGATGTTGTTGGAAGCTTGGTTTTTCTGGCAATTCGCGATTAAATGGTTCTTAATGCGGT GAGCCTTTGTTTTTATACAATATTCTGTTT
<b>TcoSat103</b>	TTTTCTTAAATTTAACTTTTAACTATCTTCTAATTTAGTGTAAATTTCACTTGAAGAATGACAAAAATAAATTTTTTCTACAGAATCTAGAAGATAGAAGTCAAAAAAT TTTTCTGTTACTTTTTTACTCTGACTTTCTGTTCTAGTTTATCTCAATTTACTTCTAATTTAAAGAAATTTTCAAAAAAGTGTAGTTTTAACTCTTTTTGCTCTGT





<b>TbrSat24</b>	AATGAATGTGATAAAAATGAAATCTGCCAATTCATATTGCGTGAAAAATGAAGAATGTGGTAAAGATATAGTAAAAATTTGGTGAATGTGGTTCAGTGTA
<b>TbrSat25</b>	ACGTTTCTCAGAGGAACCTTGTGTTCCGAGGGTGTAGTGTCTGAACCTGTGCAACTCGTTACAGTTGGAGTAG
<b>TbrSat26</b>	GGCAAAATAAAGCCGTAATGACAAAGTCCGCTGAACGTTAAAGGTTGCAATTTTTTTCATAAATAGTCTATGATATTCTAACTGCTGGTTAAGGTCTCATCTTGGC ACAAGATCGATAAGAAGTTGAAAAAATTAACACGCAAAATTTCCGGGAAGAT
<b>TbrSat27</b>	GCCCTACCGTAGATAATGCTGTTTTCGTTAGCGACGAAAGTGGTCTGACTCCAAACCGCTGGTTGAAGAGCTGCCTGAGATCCAACTTCAACCAATGACAAGA GAAGCTGTACCTGCGCTCATAGTGAATGAAATTTCTCTGCCACATACCTTGTGATATTAGACAGGTAACCTTAAATGGTGTGCTATTTTAGCTTCAATAAATTT TTTTTAAAGGTTCTTTAGTAATGAT
<b>TbrSat28</b>	GAAAAATGTGTTGATTAATTTATTAATATTCAAATTTGAACCTCAGGAATATATAAATTTGCTTTAAAAATGTTTGAAGAATTTATATTCTGAATCTCTGAGCAAAAT GTACTGAGCTACGCGTAGTAGTTTACAAAAAATTAATTTTTGTTTTTCGAAATACTGTGGACCAACTCTGTGACTACTAAATAAATAATCTCAAATGAACTGAAG AAATTTAAAAATCGCATCAAAGTGTTTGGAAAAATGTCTGTCAGACGCTCGAGGAAATGTAATAAGATACGAATGTACTTTACGAAAAATCAATTTTTTTTC ATAAGAAAAAATGTTTTTTTCTAAAAGACGCTGTAACATTTTGTCTTGTGCATTTTTATAAAAAATTAATAACAAATTTTGTCTGCAACAATAAAAAATAGCTCAA AATCAGAAAAATCATATTTTGTGCGATTTAATAAGAAATTTAGTTAATTTTTGTCGGGGTCAATAAATGACTAGTTAAAAAGTTATAAAACGCAATTTAGGCTATT TTGTACGTTTTGTTTTTATTTGAAAAAGTGACCAAAATTTGCTGTAGCGTCAGCAAAATAAATTTTCATTGCTATTATACAAAGTCTATCCACAATTTTGGTTGAA ATT
<b>TbrSat29</b>	GTCAAAATTTGATTGTGGTTTTGACTAAAAAGTTTTGCTTTTTATTTTCTTAATGGTCCATCATTGCCGATTTTTGTGCTATTGTTATACAAAAAGACGAAT CTCTATCAAAATAACGATCACCATTCTAATGCCATGATTTTTTAAATCAGTAAAAATAAATTTTAGAAAAATGTGTTTTTCATAGTCAAAAGTAATTAATAATGATAA ATACAAAAATTTCCGTAATTTGACGCGTTATGAAAGCAATTTATAAATTTTTCATTAGTTGAAATGAAATGTACATCGTCAAAATCTCAA
<b>TbrSat30</b>	CTTTTAAAGATTGACCAAAATTTGATTTTTTGTCTTATAAGATCTGAATTTTCGCAAGCAAAAGTAGGAGATGCTTACGTAGACAATGTCCTGAGTGTCCAGTGA AATCAGAAAAATCATATTTTGTGCGATTTAATAAGAAATTTAGTTAATTTTTGTCGGGGTCAATAAATGACTAGTTAAAAAGTTATAAAACGCAATTTAGGCTATT TTGTACGTTTTGTTTTTATTTGAAAAAGTGACCAAAATTTGCTGTAGCGTCAGCAAAATAAATTTTCATTGCTATTATACAAAGTCTATCCACAATTTTGGTTGAA ATT
<b>TbrSat31</b>	GTCAAAATTTGATTGTGGTTTTGACTAAAAAGTTTTGCTTTTTATTTTCTTAATGGTCCATCATTGCCGATTTTTGTGCTATTGTTATACAAAAAGACGAAT CTCTATCAAAATAACGATCACCATTCTAATGCCATGATTTTTTAAATCAGTAAAAATAAATTTTAGAAAAATGTGTTTTTCATAGTCAAAAGTAATTAATAATGATAA ATACAAAAATTTCCGTAATTTGACGCGTTATGAAAGCAATTTATAAATTTTTCATTAGTTGAAATGAAATGTACATCGTCAAAATCTCAA
<b>TbrSat32</b>	CTTTTAAAGATTGACCAAAATTTGATTTTTTGTCTTATAAGATCTGAATTTTCGCAAGCAAAAGTAGGAGATGCTTACGTAGACAATGTCCTGAGTGTCCAGTGA AATCAGAAAAATCATATTTTGTGCGATTTAATAAGAAATTTAGTTAATTTTTGTCGGGGTCAATAAATGACTAGTTAAAAAGTTATAAAACGCAATTTAGGCTATT TTGTACGTTTTGTTTTTATTTGAAAAAGTGACCAAAATTTGCTGTAGCGTCAGCAAAATAAATTTTCATTGCTATTATACAAAGTCTATCCACAATTTTGGTTGAA ATT
<b>TbrSat33</b>	GATAACCGAAAAATTCAGTTACATCTTCTCTCTGCTTACTGCTTTTTTCTTAACCTATTACACACAATTTGACATAAACATTATAAAAATAGTTTAAACGTCGAAA ATCAAGTTTTAGTGGCATTGTGACAGAAATTTGTTAATTTGTTGAAATATTTAGTTAAATCGTCTGAAAAACCTCTCAAACCGCATTAAAGGACGGTTCCTGTTG ATAAACGCGACAGAAATTAATTTAGTGAATTTAATGAAATAGACACAATTTCCACCCGAAATACCAAAAGCTCAGTTAAATGTTAGAAAAACTACTCAGAAAGC AATCAAAAGGATTTATGTTTGTACGCTGCTGGCTATGATTTTTCTTTTACAATCACAACCTACGAAATTTTCAAAAATAGTAGAAAAATAGTTTCAAAACACAACGAAT CAGTTTTAGTGGCATTATGATAGATGTTGTTTACAATTTACTCGG
<b>TbrSat34</b>	ATTAATAAATCTTCAACTAGAAATAAGCAAAATTTGTAATAATTTTCAATGTTTAAATTTGCTACTTTCAATTTCTTCAATTTCTTCAATTTCTTCTTTTATCTCTTAAAT ATCTCTTTATGTCAGCTGTTCTTTTATTTCTTCTTATGCTTTTCAATGAGCTTTTCAATCATCTTCAACATTTTAAATTTTGTGCTGTTGTTTTTTTTGATGATGATTTTTCAAT TTAGTACTTCTGCAAAAGGTCATCTTCTCCTCTCTTCTTCTCCTCATTAGTCGAGTAGTCTGTACCGCTCAGGTCAAAGCTCAAATATTATTTTTATCATGT AAAACAGCTGAGA
<b>TbrSat35</b>	TACACATAAGTGTCCATTTATGAATAAATCGGTAATAATGTTACTTAAAAAGACTCTACATATTTGTTCTGTTTAAAAACAAGCATTATTAATAAATAAATCTATAAT ATAGCTTAATGTGCAAAATAGGTTGGCTGTCTCAAATGTCAAAGTCAAATTTGAGAAAAATTAGTAAAAACGAATTTTCAGGCACATTTTTCTGAAAAACGTTGTT TGAAAAATGATTGCTATTGTTTTAATTTACGTTTACTCAAATTTGCAAAAAATGCTACTTTTATCTCAAATGAATGCTCATTCTAATGTTGA
<b>TbrSat36</b>	CATTATTTCTGTACATCGAAAAATAACAAAAAAGTTTTATTATAAGATTGCAACCAACTATCAAAATTCATTGCTGCTCAATTTATTGCTAAGAAATCAAATCAAAG AAAAAGAAGACCGTTAGTGAGATTAATAAAGTGTAAACGTTACCAACAGACTTCCACAAGCGCGGCTACGACATTGGACCTGTTTCTGTGACATCTTTGAAATA AAGACCAAAAAATTTT
<b>TbrSat37</b>	TTAAAAATAACAAAAAGTATCAATGAAGTTGTGATGATTTTGAATAAGTATTGTGCGATGAATTTACAGGAACCTGGAAGTCTAGGTTAATAAATCTGGTACCAGTCT TGCTCTATAAAGTTCTGTTTCAAAATAACATGTTTTATATAGTTTTGCGAGCTGACATGAAGTATTGTTTCTATAGTACACTTTGGTGTACAAAAAATAA TCCAAAAATAACAAAAATGGCGGCCACAAGTTCTGCAATTTCTAGTTACGATGTTTCAACTAAACAAATTTGCTGATTGAAGCTTGGAAAGTTTT ATGTAATTTGATGTTAAGTTTTGTTGAAAGTTTAAAAATCTTAAGTAAATTTAATAAATCTTAACTAGAAATGAAAAATTTCCAGGGAGAAATGAAAAAGTGGGAG TTCTATTATTTAAATATGCAACTGACCATCAACAAACTCTTCTGCTAGTCTTTACCGCAGACACT
<b>TbrSat38</b>	ATTTTTCATAATTAATGATGTTTTCTCAAATTTGCATAAATACTTTTTTATAAATAAATAAATTTTGGAAATTTGTGACCAATTTTCCCAAGGAACGGAAGGAAAA TAATAGGAAAAAATAGTTTTACATAATTTTACGAAAAATAATA
<b>TbrSat39</b>	TAAAATTTAAGGACTTTGTTGAATTTAGAACATCGAGAGTATTGACTACAAAATTTGTAGAATTTGTAAAAAAATTTCTAGGTTGTGTTAAAATTTCTAGAATCT TGATACTTTCTCAAACGTCGACACTTAATTTGTTCCCTAATTTTTTTATAAAGTTGTAAAAACTTTGAAAATTTAAAAAATTTGTAATTTCCCAATTTTAACTCAGTTGAAT TATAAATCTCCTAGAATTGTAAGAATAAATTTAGACCTTGGAAAGAAATTAATAAATCTAATAAATTTAAAGCTT
<b>TbrSat40</b>	TTGTAGTAAAAAATAAAGCTTATTGCTACGCTCACTAACCAAGTACTCTTCAAATTTGATGATTCAATTTCTCAAAAAATCACACTAGAAAAATTAATATCTC AAAAACTAGAAATTTGACAACCTCATTTTTGATATTTAGAAAAGAAGAGCCTTTGAAA
<b>TbrSat41</b>	ATTTCTTTTTCCTCAAGAACTACTAAATTTCTAAATCACTATTTCTCACTCCCACTTGTATGATTATTACTTCAATCATGACCTTTTCTATCTCATCTAAATTAGCAG GCAAGCAATAAGAGTTTTTAAATTTTAGCCGGCGCATCCACCAATTTAGTTTATAAATAAATAAATTTGAGGCTCAATTTAAAAACGACAGAACTATTTT TTTTTTATACATATGTCATAC
<b>TbrSat42</b>	TGAATCTGTAAGGAATATTAAGTTCCAGTTGGTAGTTTTGAGTAATGTCAAAAAACCAAGTCAAGTTTAAACTTAGTATTTTCAAAAAAGTTTATAATTTTGTCT GCCACACAAAAAGTTGCTAGAGTTATTTTTTCTAGACAGAAATTAATAAGTGTGGAAATTTAATAACGGTATTAAGTTTTCAGTCAATGGTATGATGATGTTATCA AAACACTGGTGTGCAAGAAAGCTGATAAATTTTACGCGATTTGAAATTTTGCCTGTGACTGATTTTTTCTGGTACAGATTTTAAATGAAGTATC
<b>TbrSat43</b>	GGAATTTAATGATTTCAAAAAATTTGTTGATTTTCCGTTAGCTTCCACTAATTTCCAGATTTCTTAAAGTTTCTCTAGAAATTTTATAATTTCAAAAAATTTCTTT GATTTCTCATCAGATTTCTCAAGTTTCTCCAGAAATGATATTTTTTCAAAAAATTTCTGAAATTTCTGGTAGATTACTAAATTTCCAGATTTCTCAAGTTTCTCT CACTTTCTCAGAAATTTCTGATTTCTAAAGCATCTCTGCAATGCTCCAAACCTTTAATAATGTCAAAAAACGCTTTTCAAGATTTCTCTGATTTCCACAGATTTCTCT GTAGTTTTAATTTCAAACTCTGACAATTTTGGAAAGAAATAAATAATGATTTTTCTTTGATTTTTCTCTGATTTCTAAAGTTTCTCAATCTGTTTTCAGTAAATTTAT TTATTTCTGTAACAATTTAACAATTTACAACAATTTATCAAGAATTTCTTATTTCTGTGATTTCTTAAAGTTTCTCAAAAAATTTGACATTTATACATAACATAAT TTCTAGATTTCTCAG
<b>TbrSat44</b>	ATACTTTATTTTTGTAATCAATTTGAAGACTCCACACAGTGTGCAATTTACAATTTAGTAAAAACGTAATTAATCTGTGCAATCCAAGATTGAGGGTCTAAAAAAG ACTACAATGTTCTCAAGTGGATATTGAGATCTACTGAGAACTCTGAGACGTGATTTTCTTTGTTTGGAGTATCTCAAAAAAGTAGTACTGTTCAAAGTTCTCACAT AGTAAATATCTCAAAA
<b>TbrSat45</b>	CTTCAATTTGAAAGATTTATGTTCTCTAAGATATTATAAAGTATAAATACAGTATAAATTTAAGCAAAATTTGAACTTTTCTACCGTATTTTGGCTGCTTTCCAG AATTTACCAATATCTTTGTTGCTCCTGTAATGTAACATATTTTGAAGTATCAAGGTTTAAATTTTATTTTATACCAATAACGACTGCGGAATCATCGTCC
<b>TbrSat46</b>	AGTTTTTATGCGCAAGTCGAAGATTTGTTTGTAGTGTATTCAAAATTTGACAACCTTTTGTGCAATAACGAGATGCAACACTTTTTTTTACAGAAATCTCTCGGTT AAGCAGTTTATAATTTGGAAGTGTAAATAAATTTGACAATGAAGTGTATTATTTAATTTTTTGTGACAATTTTGTGACAATTTTGTGACAACATATAAATTTGCTTGTGGCTTTT CATTGTCATGCAATTTGCAAAATCATGCAAAATTTGTTACTCGAATTTTCAAGAAATTTGACT
<b>TbrSat47</b>	TAAAAATAACACTAGAATGATTTTAAAAATTTGAGTTAAGTTTTTACCAATTTACAGGCAAAACTGTCTTCAATAAATACACGAAGACGCTGAAAACCGCTTTGAAAT GTGTTTAAATGAAATTTTATACATTTTCTAGAAAAATAATGCTATATTTTTCGTAATTTGCAACAACCAAGCAAGCAAGTTTGTAAATGCTGATAAAAAATTAAGTTTT CTAAGTTGCGACTAAATTTTAAAGCTTAGAGGTAAGAAAGACGGACTCAGACAGGGCAGTAACTACCCACTAGA
<b>TbrSat48</b>	AAATTTTTTACTACTCAGATTTATTTTAAACAGTCAATTTCTCAAATTTCCCAATTTTCAAAAGAATAAAGGCAATTTCACTACTGAGGGTCCACAT TCTAATCTTGAGACTGACATTTGAAAAAATGTTCTTCTGAAATGACTTTTGAAGAATACCAAGGTCATATAATGACACTTAAAGTTATAAAAAATA
<b>TbrSat49</b>	AAATTTTTTACTACTCAGATTTATTTTAAACAGTCAATTTCTCAAATTTCCCAATTTTCAAAAGAATAAAGGCAATTTCACTACTGAGGGTCCACAT TCTAATCTTGAGACTGACATTTGAAAAAATGTTCTTCTGAAATGACTTTTGAAGAATACCAAGGTCATATAATGACACTTAAAGTTATAAAAAATA
<b>TbrSat50</b>	AAATTTTTTACTACTCAGATTTATTTTAAACAGTCAATTTCTCAAATTTCCCAATTTTCAAAAGAATAAAGGCAATTTCACTACTGAGGGTCCACAT TCTAATCTTGAGACTGACATTTGAAAAAATGTTCTTCTGAAATGACTTTTGAAGAATACCAAGGTCATATAATGACACTTAAAGTTATAAAAAATA



<b>TbrSat73</b>	AGCTCCTGATTTTTGGTAAATTAGGTTTACAATTTGAAGATTTTGTTAATTTTACACTAGAATAAATTAATTATGATTCATTTCAAAATATTTTATTTTTCATCGACAAT AATTCAGAAGACTACTGATTGAAAATTAATGAGTTTACAAGTTAATTACACTAATTTACTACTAATATAGATATTTAGAAAAAAATCTGTTTCTAAGATTTACAAT TGAATAATCGTTTAAATTAATTTAATTTTATACAGAAATAGTTAATTAATGAGTCGCTCTAGACAATTTAAAAATGCTTTTTTTGTTTTCAATCAATAATATT TCAA
<b>TbrSat74</b>	TTTTTTAAACATTGTAGGAGCTTTTGAAGCTGTCTAGAACCCTGCAGAACCTAGAATAAGTAAAACTCCTAAGAATGATGTAATTTGTGAGAATCTATTTCAAATCT AGTAGAATGTAGTGGAAACATTGAGAAATTTTTCTTTAAAAATCTGGAAAAATGCTAAAACTGAAGAAACATTGAGAACCATGTAAGATTTATAAGAATTTCCGGT ATTCATGGGACGCCACTAAAACTGAAGACGTTATAGAACCCTATAAATTTGATTTTCAGCTTTAAGACTTTTTGTACAACCTAAAAA
<b>TbrSat75</b>	TTGCGTAGGCAAAATATATTCAATTTCTGAGATTTTCTGATTTCTCTAAATTTCTCAAATTTTGTAAATTTCTCTGATTTCTCAAATAATCTTTCTTGAGATTG CTTGATTTCTCTAAATCTCAAATCTCTCAATTTGTCTAAATAAATAATTTTCATGGAGGATTAATGTTTTGAGTTCTCTAAATTTCTCGAATTTTAAAAATCTCT CCCAATTTCTCAAAAAATCTCTAAACTCCGTAATTTTATTTGACATTTTGTGGTTTCTCTGTTGGGACAAATAAGTAAACATTTTCAACAAACCAAGGCTCTAAATTT CTGAGGTTTCTCGATTTCTTCTAAATTTCTCGAATTTCTGTAATTTCTCTTAATTTCTTAAAAATCTCTAAATTTCTAATACTTATCTGGAATTTGTTGTTTCTT CTCAGGACAACTTAATGTAAT
<b>TbrSat76</b>	TTTAATTTGACATTTTAAATAAACGTTTGAAGATGCAGAAAGTTCTAAACTTTTTAAAGAATCAAAATTTGACTATAGAAGATTTCTAATTTCTATGTACATATTGCT TAGAAGAATAAGTGTAACTGATTGAAAGCTCTTGGATTTCTAAATTTGATCAATTTCTAGAATCTTTCGAAATTTTGGGTTCAATTAAGTGTGCAAGACATTCTAG AATGTAAAAATTTATCACAATAATTTCTCTCTTCTGAAAAATGTAATGTATGTATCTAATTTTATCTACTGAAAGTTGTGATTTCTGGAAGACATTTTAAATTTAG CAAAATTTTAAAAAGTCAAGCAGAACTTAATAGGAGTTCTATAAAACCTGAAACCTAGATAATTTTTTTAGCGTA
<b>TbrSat77</b>	GAATATTGGTTTTGTGTTAATTTTACACAATACTAATGCTGTTTCACTTCTGAGATGTTAAACATTAATTTACTTTCACTGATCTTTAGTTGAATTTAACACGA TTATGCAAAAAATTTAGTTTTTAAATTTTAAACGCAATACTGTTTCACTTCTGAGAGTATGCTAAAGACTTCAAAAAATTAACGTAAGAATAAGAAAGAAATCT ATCAATTTGAA
<b>TbrSat78</b>	CAAAAAATCTACATACCGGCCCTCAAAATTTTTGCGGCCCTCTAAATTTTACAGTTTTCTTCAAACCTCATATTTTCGACAAAAATATTTGGTAAAAATAAATA GAAAAGTAAAAATTTTAAATTCATCAGGACTATTGAGGCGCGATTTTATAACAATAAATAACATAAAATCCAAAATAGCTACGTAACCTTTTTTAAAGTCTAATTA AAATTTACATGAATAAAAAAGTCAAAAACTAAACTTTTACATTTTAAATAAGGACACA
<b>TbrSat79</b>	TTTTTAAAGTTCTACAACGTAAGAATTTGATAATTTTATTTCTCGAAGAACTTAAATGATTTTAAAGAAATTTACAACGCACTTTTGAATTTGATGATTTCTA ATGTTTTGTTAGTTTTCTAAATCCAAAATTAATGTAATATCTACAACAATTTTTTATGCAAAAAAGTTGACATTTGCAAAAAATATAAAAAATTTACGTATGT AAGTAATTTAAAAAATAAAAAATTTTCAAGGTTTAAATTTGATGTTTTTTTTAGTTATTGTTAG
<b>TbrSat80</b>	AAATATTTCTGAATTTCTGAGAGTTTTTGACAATTTCTGAAAAACATTTGCCGAAATAAAAACTTTTCAAAATTTCAAATCTCTATTAGTTTTACGTCAGAAAAATA TTTTGTTGCTATTAGCCAGCGCTGAGTTAACAATGCAATTTTTTGAAAAACTTCAAAATTTTCCGAAATTTGAAATTTTTTGACATTTACTTAAAGAAATTTTG CCGCAGTGAAGAAACTTAAACAACCTCAGAACTCTAGTATTACGTCAGAAAAATTTTATTTTGTGCCATTACCCAGCGCTGAATTAACAATGAATCATTTTT GACAAAAAACTT
<b>TbrSat81</b>	CTACAGATTTCTAGAAAAATCTGTTTCTGCATAGCGTTGAATACTTTTATTTCTAGAAGGCTTCCAGTGTAGGACATTTACTCTGAAAAATCTAGAAGACTTATAAG ATTTCTCATAGCTTTATGATGCTTACAGAACAAATTTTAAAGCTTACAACGTTCTACATAATTTCTAGAAGTTTCTTATATCCCACTAGTGTTTTTCTTTATTGGA AGATTTCTAGAGTTCTAGAAATTTGTCATCTATTTTGAAGCTTACGATAAAGTTGATATTTTGTCTAGAAAGTTCTACAGCGTAGAGAACATCTGTTTTAAAAATTTCTG TTTTTATTATAATTTTGAAGATTTCTGAGAGATTTTCTAGATTTGCAAGAACTTCTGCAATTAATCAAATCACCTTTTTTTGAAATTTACGAAT
<b>TbrSat82</b>	CCAAACAAATTTGGAAGCTAGGAAAAATTTACCAAGATTTCTAGATGTTCTAGAAAGTTTACCAATTTTTGTGCAAGAAACGTAACAGATTTCTTAAATTTCTTTGAA ATTTCCACAGTATTTCTAGATTTCTTGGAAATTTACCAATTTCTAAACAACTTTCTGATTTCACTATTTCTAAAGACTTCAAAAAATTTG
<b>TbrSat83</b>	ACGGAATTTATTTTTCTTTTCTATAAAGCAAGACGATATTTGACAGTGCTCAGTTTTCTGACAATTTACTCAAAAACTTCAACCGTATTGTAATAAATATATAAATTC GATTTTGCCATTAATTTACATATAAAAAATTAATTAAGTCTTTTGGACATTAGTGTTC
<b>TbrSat84</b>	CCTATATGTAATAATACATAAAAAATTTAACCATCACTTTAACAATTTCTTACTTCAAAAAATAAACATTTACGTTGAAAGTTAGAAATCTAAAAATTTACTAGTCTCTG TGAATTTACACTGGAATTTTACAATCTTGTCCAACGATTTTCCCGGACGCAATTTTTTATCTAAATATATTTGATTTTAAAAAGTGAATGTTTTTACGAAGATA AATAAAACCATGTAATAATACATCA
<b>TbrSat85</b>	AAAAAAATGTTTTTATAAAAAATTTCAACAAGCATGAGAAAAAAATCAATTAAGATTTCAATTTCAACGTTTGTGACAAAAAGAAATTTCTTATTGATTTTTG AAATTTTAGTCAGGATTTTTCTCGTTTTCTCTCCGCTGCTCTCGCGATGAAAAAAATCTTTAACAAGAAATGAAAGCAACGAAAAAAGAAATTAATTT TTGGAATTTAATATTTTTCTAGAACTGGAATTTTGAATGTTTTGAAACGCGGACTTTAAAGGTTATAAATGTTTAAATAAAAAATGACAAAAAAGTCTGCTCTCTCG CAAATAAAAAAATTAATTTTTTAAACAAAAGATAGAAGCACAATAAATAAATGATATAAAGTTAAATGATGTAAGAAATTAATTTTCAATTAAGGCAACCGA GGAATTTCTGCTCTCGCCGAT
<b>TbrSat86</b>	AAGAACTATTTTATGTCGAGAAAACTGAAGTTGTCAAATTTGACACTCTGAAACTAAAAATTTAACTCAATATAAATGAAACGTTTATAATGTTAGCAGGATTTTA GTAAGTCTACGTAAGAAATATTTAGAACAGTGTCAAAATTTTACATTTGACATTTCCAGATGACTATAGTCACTAAATATTTGACACTCTCGAAATTTCAATGTTTTCTA TAAACTAGGAATATCTGACAAACGCTTTTTTTGACAAATTTGTTTATGATTTAAAAA
<b>TbrSat87</b>	ATAGACCTCCACTACTTCTTCAAAAAATTTGAAATTTCCAGGAAAGTTGAAACTTTCTGAAACAAAGAAACGCTATGTAAGGCTATTTATTTTTCTAACTCG AAGTTTTAATATATTTCTAGAACTGGAATTTTGAATGTTTTGAAACGCGGACTTTAAAGGTTATAAATCTGGAACCAAGGTTATTTTGAAGATATAAGTGGTTC GGTACTCATGTCGAGGTCATATTATACAACTTTGGTCATAGCTTAAAAAAGTCTATAACCAAACTTTTTGTTCTAGAAAGCTCTGTAGAGTTGCTAAGTAATA GATTTAGGTGCTTAAAGTTCTACGATATTAGAAAGCTTATTTTCACTTTTCAAGATTTCTACACAGGATTCAGAGTCAGAAAAGCTTTTTTTTTAGATACTTAA AAA
<b>TbrSat88</b>	TCAGCGTTATTGATTTCTTATATTTAGAAGAGGAAATTTACATTTTCAAGATATCTCAAAACTAGTAGCCATAGAATAAATAAAGCAAGAAATTTGTTGATTTG ATACCCGAAACTTATGTTCACTTTTATATCATTATTTAAAGATATGGATGTCACATTTTTCGTTAAATAAAAAATGATTTGTTAAGGTATCTCAAAACTAGTATCAC GGCTTAAAAATAATGCTAAATTAGAATTTCTATTCTAGTTTGAAGCAAAAAAGTCTTTTT
<b>TbrSat89</b>	TTGGAAAAATTTAAATAACGTTTTCAAAATATTTCACTTTTTCCCAAAATTTTAAAGAAATTTGAAATTTGACTTTTAGGCCAATTTACTTGTATTTTTAGAATCTGGTTTAAATTTG TTCTAGTTGACATGTTTTGAGCATGTTTTGAAAAATATATACGAAAAATTTGGCCACTACTGCTGATGACAAACGAAACATTTCAAAAACTACAGTAACTTTTATTTTA TTGAAAACTTATGTTGCTGTTGGCAGCTTTAGTTTCTTGCATTTTACATAGGAAAGATAAAAAATCTATTTGTTCTGCTGTAATTTTTAAAAAATTA
<b>TbrSat90</b>	TCCAACCTAAACGGTAAAAAGTTACCAATTAATAAATAACGAAATCGATAAAAAATTAATGACTTTTTGGGAAAGATTCAGTTGTTTTTCAAAATTTCCGTAT CAATATCGGCCACATTTGACCCATAATTTTATGCAATTTAATAAATACTAAAAACTGAATTTACATAACCTCACTTTTTTTCGACCTTTTATGACAGATTTCAAT GTT
<b>TbrSat91</b>	CGTACTGCTTTGTTGTGTAAGTAGACGATGACTAGTTGGCGTATCGGACGACGG
<b>TbrSat92</b>	CGAAAGGATATGGTAATGGTATTTTTTGTCTTCAAACTGTTAATTTGAAGAAAAAACAACCGCACTGTTTATACCTACAGTCACGTGACACTTTTTGACATTGACA TCTGTCAAATTTCTGTGTTGCTGCTCAAAAGTGTGTCATAAAGAACACATAATGAGATCATTTTGAATAATTTGTAAGTGTCTAAGTAAATCTTCAAAATGGGCT GTTTGGAAAAACCC
<b>TbrSat93</b>	TCACAATAATGTCGATTTCAACCTCACTTTCTGCGACAGAAATTTACTCACTTTTTATTTTACCCTGAAGTTGCTTTGATAATTTTAACTGACGCCATGTTACT TTAACTCTCACTGTTCAAGAATTTGCAACCTGTTATGACAATATCCCTACTAGTTACTGCTGATCATCTTTCACTTTGTTTAGGAGGAATGGTGAACAAACCACTA TTTTCTCCCAAAAACTCTGCTCGAACT
<b>TbrSat94</b>	TTTTTCTAGAAATGGTAAACCGGACGCGCTGTTTTGTAATATATCTTTGAAAAATTTCAAAAAATTTTACAATACCTCGAAAAATCAAACTCTTAACTTTGAAAAAGTGT TGTTGATTTTTTTTGAATAAAAAATTAAGCCATTAATAATCTTTTCTAAGCACATCAGGAACTCAGTAGAACTCTCAGAAAGACGTATATTTATCTAGAAATTTCT GAGAACCTTAAAGAACTGTTGTAGAAAAAGTCAAAATTTTTTGAAGAAATTTTCTGGAAGAACTCTCGACG
<b>TbrSat95</b>	CAAAATGTTAATGTTCTCTTAAACGTAAGTACTACAACATTAATTTCTTAAAAATCTAGTTGACAGTTTGTACTTTTTTATTTTACATGTTCTGGT AGTTACGTCAAAAATTTCAAAATTTTAAAAATTTTAAAAAAGTGTACTATTATTTGTTGTTTTTGAAGCCCTGTAATGATACAGAGACTTTAAAGACGCAACATAA CACTTTGAAAAATTTATACAACATATAAAATTTAGATTTCTCAGGATTTAAAAATTTTCAAAAAATGCTCAGGCTGTAACATTTTCAAAATGGATTTACAATTTAG TTTT
<b>TbrSat96</b>	TACTGAAACTTCAACACCCACTGAAACATCAATAACGACTACTGAAAGGCCAACACAC

<b>TbrSat97</b>	AAAACTAACGTAATAATGATGATCGAACCTGAACAGACGACCTGACCTGACCTCTCTAAATTTGAATAGTTTCGATAATTTCCAATAATTACGATTAATTTCCGGAAAAAGTTAGAAAAACCTTTTATATTAGTTCCGAATAACTTTAGAAAAACGAAAAACACACGTGAGAAAAAGAGTATTTTATACAAAAAACCGCAATTTTCGATCTACAAAAATTAACAACTCCAAAACTTGAAAAAACAAAAATCAATAACTAAACAAATTTACCAATAATTTACCGTAAAAACACACCATCACACAACACACTTCGGCCGCTATTGTCTATCCAAAAATACAAGAAAAAGAAAAAGGAGAGAAAAAGAAAAAGTACGAGAAAAAGAAAAAGAAAAAGGAAAAAGAGAGATGGAAAGAAATGAAAAAAGAACAAATAAACGACTCGCGCTTCGAACCTGTAATTTTCGGATGTTTCGGTCTCCGAAATAGCAACTTCGAATTTGATATCTCGCTTAAATTTGAGATATTCGAAACGGAGTTGCATATAAATCCGAAACGCTGAAAAAGGAGAGATTAACCACTTTAAAAATTTGACCTTGAAAAATCTATCTCGGTTATTTGAAGATATCGGAAAGCGGTTTTTCGCGAGATCGTAGAGGACCTCTAGTTACGTTTACACTTTTTACCGCGTCAAAAAATTTCAATGGGAAAGCCGTAACCAAGATTTCTAAAAATCTCGGGTCAAAATTTCTCCGTGTTCTGGGAAATATCGAGCCGAAACCGTGCCCAATCGATTCCCAATGGTGCATATATGGGTATATATCGACTGTAAACAGAACCCGGAATGACCTGACCTGACCAATTAATATTGATCCAACCT
<b>TbrSat98</b>	AAGGATAGTTTTTTTCCAACACTCAAAAATTTACCGAAAAAGTTTCACATAATTTTCAAATTTGGAACCTTTGATAATTTGTATAATGGAAAGTACACCTTCGACCCATTTACTCATCAGGTGCTAATTATCATTGATTTCTTAAAAACATCTGGATTTTGAAGAATTCAGTGGTAGTTTCGACGATTACTAAAAATTTAGGATTCAGCGTCAAGGAGTTAAATATTCAAAATGCTATTTAATTTTCACACTGTTGCACTAATAATTTGGCAACTGCCAAGGATTTTCGAAAAAACATTTTTTTGAGTCG
<b>TbrSat99</b>	TGTAATTTTACGATAAAAAATTTGAAAAATACATCAATGTTGGAGACAATAAAGATGTAATTTTAAAGATGTCGACTGATTTTAAACAATTTGAGAAATATTCTGTTCAAGAAATGTAACACTACGTAATGTTTCAAAATTTTACTTTTACACAATTTTTTACTCGCTGAAAAATTTAGGAAAAAAAATTTAAATATATCATGTTTCAATTTCCATAAAA
<b>TbrSat100</b>	AGAGATTAATTTCTATTGACGAGAAATTTAAACAAAAATGCTTGTAGTAATTTTGAAGATATCCAACAGATAATTAATCTAGAGAACATCTAAACAAAGAAAAATACAAAAAAAGATGTTGAAGTGCATGCAATACAGAAATTTTTCATCAAGAATTTGTTACTAAGAAATTTTTTAAACCAAAAGTCAAAAAATTTTCAGAATTTTTTTTATTGTTAGCATATTAAGAAACATTTGTAACCTGAAAGCATTACAGAAATGATAAAAAAGTTGTAAGTATTTTTTAGAAAAACGATAATTTACGTTTACAAACCAATAAAAAAAGATATAGAAATTTCTCAATAAAAAATTTTCAAATGAGGAAACGGCTCATAGAAAGATAGATTACGAAATATTTCAAAGAACATCGTTGCAAGTATGATAATTCGATTGCAAGAATTTCCATCATAAAAAGCTATTATAGATTTTCAGGAGTAGAAGGTAACAACTATTGTTTCAGCAACTGAA
<b>TbrSat101</b>	AAATAGCAATGTTTTTTGAAAAATGAGGACGCAAAAACTTTTTTTCATTGCTTCAACTCAATCAAAATTTATTTTAAATGTCAGTATCTGCAAGTTTTATACTACTAGAAATTTAAAAATAGCAAATTTAAATGAGATAGCTAACAGTTAAGGTTCTTTAAATTTGACTTTGCTTTCTGATTTTCTCGTCAATCAGCTTTCTGAAATAGTAAATTCAAAAATGAGGTTGTAATAAATGTTTTTCAITTCACAAACGTCGACAGATTTTTTTTAAACA
<b>TbrSat102</b>	AGTAGTTTTGAAAAATTTGATTTTGTATGTTGCAATGTTGCAAAAGATTTTGAACACTCTGTGAAAAATCTGGTCAAAAAATTTGGGCACTAGTTTACGAGAAATGATATTTTGTATTTGATTCACTGTAGATCCCAAGGGTCGCAATAAAAAATTTATATAAAAA
<b>TbrSat103</b>	TTCAAGGAATGATCTTTATTTTCTCCACAGAAGTACGAAACCTGTTTATAATATATCAAAATAGATGTTTGTAAATTTTGGTTTTGAGATATAAAATTTTTTGGCTGCAATTTTAAAAATTCGACTGTTTACAGTATTACAAGACAGCGATTTTAAATGACTGG
<b>TbrSat104</b>	TAATGACACGAAACGAAAAATTTGATAGACAAATTTTGTATTTTGTCCACGCTTTCGAAATTTTGTATTTGATGTTGTAACAAGACACAATACTAATCGGAAAAATTAACGTTGATAAAGATTATATCATGAGAAATCTACGTTTCTATTCTGCCACCAATGATACATGTCGTTGAAATGATAGGTAATTTTGTATTTCTTCCCCCAATATCATTGATTTGATAGACAAAACTAGTCGAAAAATTAATGTTGATAACAAAAATATATCATGTAGAGTTCTTTGAGTTGATTTCTGCTGACTTTGAGTATTACAACTTTAACCC
<b>TbrSat105</b>	GTCAATGGACAAATTTAGATTTAAGATGCTTCTAAAACTGATTTTLAGAGTGCATAAATTAACAGTTAGTTGCAAAATGAAAAATCCCGTTTATTGCAAAATAACAAAAATGACACATGCTTCAAATGCAAAATTTGGCAAACTTCAGAAATTTGAATTTACGAATCAATCAAGACCAAAAGTCTCTTTTCCAGCGCTCCCTTTTAAAGTATTATTTGTTCTTGTATTTAAATAAATTTTAAATTTTTCCTCAATCAAAGCTATAAAAACTGAAAGGTGATTCCTACTTATTAAACAAAAAAA
<b>TbrSat106</b>	AACGTTCAAAAATTTTCAAAAAATTTCTGATTTTAGTACCTAAGAAATTTTGAACACTCTGTGAAAAATCTGGTCAAAAAATTTGGGCACTAGTTTACGAGAAATGATATTTTGCATAAATTTTTCGAAAAATTTTATCATCTTCGAGTGCITTTCTGTAGTACTACTATTTTTCACAACCTATAGAAAGTTATAGAAACTTCAGAAATCTCGAGGAACCTTTTCGATAAACGTTCTCAAAAAATTTGCTAGTGTGAAAAATAA
<b>TbrSat107</b>	TAAAAATACAGGATTTAGGCCATTTGCAATTTTATTGCTTATAGAATGTCATAGAAATTTATAGAGCTTTGTAGAACAATCAAAATTTAAAGAAAAATGCAAAACCTTATTTTTTAAAGTACTTTGAGAACTTTTAAAAAATACATAGTACTGACTCTGAGAGTTTGAACAATTTTGAAGAACAGACAAACGAAAAATTTTCAATACTACATTTATTTTTGACTGCGAATTTTCTGGAAATTTGAAAAAATCTATAAATCTTATAGAAATCTTTTGAATTTTGAAGTTTACACAACTTTAGAAATTTTATGAAAAA
<b>TbrSat108</b>	GAAAAATAAGAAATAAGAGACATAATTTTCAAAGTAAAAAATTTGCTAAATCTTAAAGTCAATTTACGCTTAAAAACATTTTGTGAAAAATGTAAGTACTGTCAGACTGTCAAAAGTTTCAAAAAATCTGAGAAATTTGACATTTTTTTCAGTGTAGTGAACACATGTTGCAAAATTTAAATTTGCTTATTTATGCTTGTATTTTAAACCAAAAAAATTTTCTCCTTTACTACACTACAAATTTTTTTTCTATTCAATATCTTAAAAATTAAGCCTTTCC
<b>TbrSat109</b>	AAATTTATCTTTGGGCATTTATGACGTACAGTTAGTGTGCCAACCTTCAGGAAATTTCTAGATTTGGGAAATTTAGGTTAGATTTGGTATTTTGTCTATAAAGCTTCTGAGTTGATACAGATTTTGCATTTCTCAATTTTTGGTGTACAGATTTGCAAAATACACTAATTCAGATTTCTATATTTTGAATTTGTTTACGTCATCGGATCTTTCAGTCTGCAAGAAAGTTTAAATAACAAAAAATTTTCTACGGCGAAGAAAGTGCAGATTTGTTG
<b>TbrSat110</b>	CTAAAAATCTATGCTATTTGTTTATTATGTTAAATCTATTGGGCATTTCTTAGAAATCGGTAAAAACCTTGTGCAAAATTTGAGAAATTTAAATTTGCTTAAAGAAAAATATTTTTCTTATTTAAATCTGTGAACAGTTCAACAAAGAGTTAAAGTTTTTATTCTATTGCAATTTAGTCCGCTAGAAACTGTGAAACTCTAGAAATGTTGAGAAATTAATAAACCAAGCTCTTTAGCCAGAATTTGTTAAATTTGAGAAATTTGAGAGTCTCAGAAAGAAATAATTTATCATATAAAATCTTAAACGCATGCAACGAACG
<b>TbrSat111</b>	ATACGTGACGTTAATTTTTATTTCTTAAAAATTTGCAAAATGCTTCAGGGTGAAGTCAAACTTTCAAGTATTAGACAAATCACTAGCAATAATGTTTGTGCAAAATTTCAAAATTTGCAAAAAACAAAGTCTGCTATTATACTCTTAGAAAAATTAACATCTGAAATTTGCAATTTTAAAAATTAATTTTTTGCACATAGCAAGATTTAATCGAAAAATTTAGCTTTTGCAGCTAAGTATTTACAGTAACTGATTTAACAGTAACTGAAAAATTTTAAACAAATTTTAAACAAATTTTGAACATTTTTAGTTTATAAAATTTTCCAAAAATTTGACATTTTAGAAAAAAATTTGACATTTAGAA
<b>TbrSat112</b>	CTCTTCATTCTTGCAAAATCGAAAAACAGCAAGTATGATGCGGATAAATGAGTATTCAAAATAAAGAAAGTATTTTCGGGTGTGCCACTGGGTTCAAGTGTGGGGCCAGTACTTTTTGTTGATGGCAAACTGTTTAGATAAAGAAATTTACCGTTACCAAAATTTACCAAAAGAAACGAAATTTGAGGATGCAATAACATTAATAAGATAAATTTAGAACGTAATTAATAAATTTATAAATAAATTTACCGAAGATATAAAAAATTTAAATAAATTTGAAATTTTGAAGATGTCAGATGCTAAGGAAGCTTTGATTAATAAATTTATGACCTGGGATAAAGAGTGTGTTTTAGCTCTGAA
<b>TbrSat113</b>	ATAAATTTCTATGATTTTTCTTAAAGTTGACTAGGATCTAGAAGATTTCAAATGTTTCTACAACACAGCAAAAAATTTAGTATATTTTT
<b>TbrSat114</b>	TAGTGTCTCACTAAAAATCTAGAATCGAGTAAAAATTTTAAATTCGTGCAGAAAAATCTATTTTTGAAAAATATAAGTAGTGGGCAATATAGAAATTTCTAGAATGCTTTGGAAACTTTCAAACGCTTAGAGAGCTCTCAGAACCTCT
<b>TbrSat115</b>	GAGTAAATGCAAAAAAGTAAATTTTCAAGAAATTTGTTGAAATAATTCATAATTAAGATATTTCTGATGATAGAAATTTTAAATTTATATTTTGTAGTGGGAAATTTGACGAGATTTTAAATTTTGAACCTGTTAAAGCCAACTTAATAAATTTTGAACCAATTAATAAATTTTGTGACTAATTTGTTAAAAATTTATGAAAAACCGACATTTTCTATTTTTTTGTAAGCCATAGTTGAGCTATTCAGTGTAGAAATTTTCACTAAGGTTTTATGTTGTAATAAATTAACGAAATTTTAAATTTCTAAAGTCAATGACTACAATCAGTTGTTTCGAGTAACATAAAAAAGTGAAGTAAAAATGGACACTTTTTTAAATATAATTTGTAACAGTCAATAAATAAATTTCACTGTCAAAATTTAAATTTAATAAAGCCATTTGAGTAAAGAAATTAACGAAGACCTCAAATTTGCAAACTTAAAGCCAAAAATTAAGCTTTTTT
<b>TbrSat116</b>	GAATCTTTGACAACTGTCAAATATATGGGAGACTACCAAGTATTTTATTTATGCTAATTTAGGACAAAAGTTATTTTAAAGCCAAATTTTAAAAATTTTCAAATTTTGTCAAATTTGCAAAATATAAATGCTAAGATTTTATAAAAATTTTAGCAAATTTAGCAACACAAACAAACCGGTTCTCAGAATTTTAAATTTTCAAGATTTGACACTGGTGAAGATACAGTGTCAAAGATTTTCAAATGCTAATAAGTAAAGTAAAACAAAGTTA
<b>TbrSat117</b>	CATTTAGAAAGATAGTTGGATAAAAAATGTCAAAAGATTTGCACTTCGAAATGATTAATAATGTAAGAACTTATGGAATTTGGGGAAGATTTGAGGTTGCAAAAGACGAAATAAATAACACGAGTAAATTTCAATGTTGCTATTTCTAGATGTTT
<b>TbrSat118</b>	AGAAAAATAAATTTCAACTGTAGAATATACAGAAATTTGTAAACTGGCAAAAGTTTTATTTTTTATTTAGATATAGAACAGTGTAGAATGTTATACTAATTTCTGAAACAAACAAAGATTTCTTTATTTTCAGAAATGAAAAAATACAGGACATGTTTTTAGTCAAAATGTAGAACTTTCTGAAAAATTTTACAAATTTTATGCAAAAAATTTGAATAAATTTAGTAACTTTGAAAGATTTGGAAAGTTACATAAAGTTTTTCAAATTTTGAATAATTTTAAATAATTTATAGAAAGTAAAAATTTCAACTGTAGAAATTTACAGAAATTTCTGAAAGCCAGACTAAATTTTGTTCATTTTAGAAATATAAAGTGTAGAATGTTTTAGTTATTTCAAATCAAACAAAGTTTCGTTTTTTATGAAAAATAAAAAATTTATGGGGCATTTTTTATGCTATTAGAAATTTTAAATCAAAATTTGGAAGACTTTCTGAAGACTGTTTACAACCTTTAAACCAAAAAATTTAAATAAAAAATGCAATTTTAGTAGAATTTGGAATGTAACAAAGGTTTCCAAAAATTTTCAAATTTT
<b>TbrSat119</b>	ACATTTTAGCGCAAAATTTTCAACATTTTTTAAATAAATTTTATATTTTAGCGTACTTTAGCTCTTTTATTACTTTTTAACTTTTTTAAAGTGGGATACAATTTTACGTCATGCTCGGCATACTTTTTAAATTTTGGGTGTAATTTTGGCTGCTTTTACGTAATTTTCTACTTTTTAAATAAATTTTACCCCAATTTTTACATTTTCAGGTTGAAATTTGCC

	TCATATTTTATAGTTTTACATTTTGGTAGGTTGTATATTTAGATATCATTTTACCTGTATATTTGAAGTGTATTTTACCTATTTTTCGGCGTACTTGTCTATTTTAA TATTTTGAGCGTAACCTTACTCCTTTTGGCAAGAATGTCTCAATTTTGAATAATTTTACCAATTTTATTTTATTTTACCTATTTTCAAAATGTAATGTACCTCATC TTTTATCGGTTTTACATTTTGTAGATATGACTTAATTTTGTACTTTTTTACATCTTTTAAAGGTTATTTTACCTTTTTTATCAACTTTTA
<b>TbrSat120</b>	ATTGACGGTAGGATTATTAATTTTATAAGTAAATAAGTCTATTGTTTTATTATTGTAATCAATTGTGCTTAAAGTGTGCAGTAGTGTGCTAGCATTTTTTCCACA TTTTTTCTTTTTACTATTTCTAAAACAGTAAAAATGCCCCGGAGCTAAAT
<b>TbrSat121</b>	GTTGATAGTACGATTTCAAATTTAATAAATATGTTGCTGTTTTAATAGTGCAAAATAATGTGCTAAAGTGTGCATTATAGTCTATTCTTTCCAAAATTTACTC ATTTTTAAATACCCTAAAACGTAAAAATATGCCGAATGCGAAGTTGGTAAAAATTTCTAAAATCTCTTACTATTTTTATTATTGTAACAACACTGACTCAGGGTGTG CAGAAGTATTACCATTTTTTAAAGTTTGTCTATTTCAAACCTACCTAAAGCATTAAAAATGCCCCGGAGCTAA
<b>TbrSat122</b>	GCCCTAAGCCGATAAAAAATTTCTGTAGTGATAAGTGTTCAGGATAAAGATTTTTCTCTTTCATATTTTTCTAAATAAAAAATTTTGTCTCATCTAACGTTAATCCGTGA ACGTGTTGAAATTTAAGACAGTGCAGAGTAAAAAATATTTATGTAAATGGAGGTCATATTTACGGGCAATTTTGACATGGCAGCTTTTGTCCGGCGAGCAA ATGACCGTACACTTTAAAAATGAGAGCCTTGAACAATAATTCGATTTATAAAAAACTGTCTTACAACAATTTATTCAGCAAAATTTCTGTGCCAAAACAATCACA AAATATCT
<b>TbrSat123</b>	CACCTTCCACCGAGTCTAAAACGATGTGAAACAAATTTCTTTGTTTTACTTTTACAGAAATTTTACTTAAATATACTATAAATCGATAGTTTAAAGTAATACTATAGTAAT TTTACTTTCCCTGAGTCTAAAACGTCTGAAACAAATTTTATTTTACTTTTACATATAAATGAACTATCAAATATACCCTACCATAATTTGATAGTAATAGTATAGTAAT T
<b>TbrSat124</b>	GATCTAGAACTTATAGAAATTTTTATTGAGTGTAGACTGTCTAGAGTTTACAAAAATTTAAAACTTGGTAAAAATTTCTACAATTTTTGAACTCGTTCAAGTTTTTA AATCAATTTGAAAAATTTTTGTATATAGAAAGCCTTATAAATTTGTATAAAGATCTAGAATGGTATAAATCTTTAGAACTTCTATAATTTTACATTTTTGAAAAAT CTTGCAAAATTTTCAAAGTGTAGGACAAATGCAACTTTTGAAGTTTTTGGATCATTCTGGGCTTGAAGACCTTATAAATTTGTAGAAA
<b>TbrSat125</b>	AAAAAATTTTCTCAAAGTGTCAAATATAAAGTCTTGAACAATTTTTCTTATTTTCTCAAACAATTTTAAACCCTTTTGCATTTATCATGAGCAAAAAAAGCTCTAAA ATACTTTGGAAAAATTTATAAATACCTTGTACAATATCTGTAGTCAAAAAATGTTGCAAAATACGAAAAATTTGCATGGTTTCTGTAAATAAAAATGTCAAGCATT ATTTGAAAAATTTCAAAGTGTAGGACAAATGCAACTTTTGAAGCTTGGCAATTTGCAAAATAAATTTTATTGATTACAATTTGCAAAATGCAAAATGAAATTTTGT TTAAATGTTTAAAGATAAAGTGTCAAACGTTGGGAAATATCGTGAAGCAATAACTGTTAAATACAGTTATAAGATTAAAAAAGTGTCAAATATACATTATACATAGT AGTTTCAAATAGTATAGTATAGTTTTAAACAGTTGTCAAATTTGA
<b>TbrSat126</b>	TTACCTTTTACAAAAATTTAAAGTAAACATTTGCTTTTTAAAAATGACAACAATTTGACTGTCAGATAATTTAACATTTTTTGAACAACATATCTGTTTTAATTTCTTTT TTCCGCAATTTTCAAAGTCAAGTGTCAAAATATCCAATCTTTGACACAATCTTGAACAAATCTTGAACAAATGCAAAATGCAAAATGCTGCGAATTTTCAAAAAATTTGACAGTCAAAT TTAACATCTTTAAATCAAACTTGTACTTTTGAAGATGTTAAAAATCTGACTCAAAAAAGTCAAATTTCTAAAATGTAACATTATAGATGCTTTAACACTTCAAT ATAGGTTTTTGAAAAACTGTCAAATTTTGTATTGACGAAAAATGACAA
<b>TbrSat127</b>	AACAATTTTAAAGTTTTTGAATTTCTAAAAAGTTCTTGAAGTTCTACGGGCAATTAATAAAGAAATCTTTTTTGTAGTAAAAAGCAATTTTGAAAAACTTCAAGAATC CTAGCAATCTTGAAGATTCTGAAAAATTTTGAAGCCTGAAATTTTTATAGGATCTATAGAAGCGATGTTTTTTTTGTAGATCTCAAACTTAAAAAAAAGTGTATA TTTTATGAAAAATTTTCAAGGATTGAGAAGCTTCTATACGATTTTGGAAA
<b>TbrSat128</b>	CGCGTGTAAAAAATAAAACCGGTCAAGTGGTTGACTTTTTATATTTTTGTTCTTTTTCTGCTATTATCAATTTGTCATATTATTATCATCAAATTAAGAAGTTGAG TACCATAGCCGTTGACGTCATATGTATCAGTGTACGAGATTTCTGACAAATCTTGAACAAATGCAAAATGCAAAATGTTGAGGCGCAATCTGGGCTTGTAGCAAAAAATACCATTGTTG CACTTTTTGAGAAATAGGTAAGTCAATTTTCTGATTTTTGTTACTTTTTCTGCCATTTGCAAAATTAAGAATCTCGAAATAT
<b>TbrSat129</b>	AACATTTTTAAACAAGATAAAATTTTAAATTTAGACGTTTAAAGTAAAAATGACCAAAAAATTTAAAAATTTAGGCTCAAAATTTTCAAGTAAACAGCAAAAAACA GTTTTTGTAAATTTTGAACGTAAAAAAT
<b>TbrSat130</b>	TAGTTTTTAAAGTTTTTATATAGTGGCAGAAATGATAGTAAATGTCAAAAACTATTTTCTACTTCACAATCAGTACGAAAAATAATTTGATGCCATACATTTTTTCAAACACTA CACTTTTTGACAGCACTTTTTCGAAGTTGTCAAACACCACTATAAAGTGTCTAAAATTTTCAACTTCAAGTGGCTTAAATTTTTCAAACACTTTTTCTGAAAAAGTGTCTA AAATTTGTAACAGTCTTTTTTGTAAATTTTCTCAAGTTTTTGTAAAAATTTTATAGAAAGTGTAAAACTTGAACAAGTGTAAAGATTTAAAAATTTGACACCTCTGAAAA ATAAATTTAATCGTGCCCAAAAAAGTTAATTTTCAAGCAATAAAGACAAATTTGCAAACTTCAAGAAATTTGCAATTTTAAATAGCCTTTCAAACGACGTGCGGTGT CAAATTTGCAAAAAATTAATGCTATTTGAGGCTTACGAAAAAGTAAACGGCAACTTAAAACTTTTTTTTT
<b>TbrSat131</b>	AATTTTCTGAGCGTTTTCTCAGTATTCTATTTTTTAAATATTAGAAATTTCCGTAATAAACAACCGCAGGCTATGTTTTAGAAAAATTTTCAAAAAATTTATTG AGTCTTAGGACGATTTATACATAAACTCTATAGAAGAAATGAAATTTAGAAAAACTTCAAATTTAGTGAATTTTAGAGTTTAGCCCATAGTTGTAATAAATTTT CTTGAAAAAAACTTTTTGTAGACCGTTTTTACAACGGAGAAATTTGAAACAGTTTTTAAATTTTAACTCTAGAAACTTCTACTGTTCTACAAATTTCAAGTTCACTCTACATC CGTAAGACATTTCAAATTTTTGTTTTTTCTAAAGTTCTAAAATTTGCTATGCTTATCTAAAATTAGAATTTTTGTGAACCTTCCAGTCTAGTCAAAATCTAGATGC GTCTATATAATTTTTTGTGA
<b>TbrSat132</b>	ATCCAGCACTTCTACGGAACCTCTAATGTTTTAGATGGCTAGAAAGTTTTGTATATGGTTCTAGGAATCTTCTAGTTTTACCAAGTTTCTGAGTGTCACTCAA TTTTTATAAGGTTCTGGGCTGTGTTCTCATAGGTTGCCAAAAATTTGCGTTGAATTTCCGAAAGATCTACAATCTTCACAATCTTCAAACCTTGAACAAAAAT ACAAGAACCTAGATTTCTATTACACTATCTGTGATAGTATCTTCAACAAGTGTCAAAGGCTTATGATATTCTATAAAT
<b>TbrSat133</b>	GGCAAAAAACCATCTTTGAGTTGAAAAATCGGAAGATCATTGCAACAAAAAATTTGGTACTCGGTTAAAAACAATAAATGTTTTAGAAATTTTCTTGTGTAA AATTAACAGGATTTTTAAATATGAGCGTTAATTTGAGGTACGACTACTCTTTGAGTAATTTGACATAATACCAACCAATCTTAAAAAATAAATGTTTTTGTGCTGG TACTGCTCAGTAATCAATTAATATTTAAAAATTTGTTTTACTTATAAAAAAGTAATTTGCGGTTTTAAATATGCAACCTTTTTTATTCTGGTACAATTTTTTGTAGT AGTT
<b>TbrSat134</b>	GTAAGAAAAACATGATTTGGTATCATATATTGTAATAAATTTGAAAAATCTATATATCCAATATCTTTTTTGAACCTCTGTAATCAATCTTGGCTTTACATAT GCCAATTTGAAAAACTATTATAATCCAATTTTTGTATGATATTGTATCTTATAAAGCGCGCTTTTACTAAAAATCATTGTAACAGCCCGCAGTTTGAACCTATGT TTACGTAATATTGAATTTCAAACAATCGCAATGTTATATGAACAACATTTGACAGTTTTCTACAATAATTTAATATTGTTGGCCATTATAAATCTCT
<b>TbrSat135</b>	TCAGTTTTTGAAATTTTGTAGTCTCAACAGATTTGTTAAATTTGAAGTCTATATTTAATTTTACATAAAAAAGAGACTGATTTAAAGCCATTTAGGTGTAGGGCAC TAGCATCTACCAGTACATTTTTTAAACATACGAAATCAAGAAATTTTTTAAATTTTAACTTACGCAAAAAATCCCTCTAGTACTTATTTGCAACAATTTGCAACAAGGAC CCAAATTTTTGTTGAAATTTTTGATTTTGTAGTTCAATTTTTTATTGAAAGTCTCAATCAATCTACATAAAAGGAGAGTATTAAAGCCATTTTACTCACAATTTCTAG CACCTATGGAAGTTTTCTTGAACCTGAACCTAATTTTAGAATTTCAAGAAGTCTTCTAAAGATTTACGCAAAAAATCATTATTTGCAAAAGTTTCTAAATTTCTAAATTT AGAATTTTTGGCAATTTGCAACAAGGAGTCTTATAAAGACATTTTTCTATTATTGTAGCATCTACCAAAATTTGTTTGAACAGAAATTAATTTTTGAAACAAAGG G
<b>TbrSat136</b>	AGATTTATTACCAGTTTAGATTTTGGTTACATCGTTTTAGTAATGTCAATTTAGTACTTTTTAATCTCAAATTTGACAGTTTTTGAACCTTTTAAAGTTTGTAGAGTCAAGTT TTTTTGTATATTAATCTACAGCCATCGTAAATCAATTTTGAAAAAATAATTAAGACATAAAATCATTAAATTAACAAAGTGTGAGTATTGACAAATTTTTGATCTT TGAATTTGTTGACATTACAATAATATTACTGGAAT
<b>TbrSat137</b>	AATTCAAATCAAATAAAAAATTTACTGAATCCTTTTTAACTTCTAAATCTTCTCAAATCGCACTAAAATAGTTTTGAGCCAAGTTTTTATTATAACAGGTAAAAATA TTCGTTCAAATTTTTGAAAAAAGCAAAAAACTGCAAAACGCAACTAAATCGGATTTTGAACATTTTATAGATTATAATCGTTATTTTTTCTAAATTTTTATCTTAGA AGCAGAAAAATTTGCTCAATTTTACAATAAAAA
<b>TbrSat138</b>	TATTTTTTTATCCATCAACCTCCACATCCATCTTTTCAACAATAAAGACACTGTATCTTGAAGAATGCTGTTTTGATTTTCGATGGAATTTGCTCAGTATATTCAAAT GGCTTTCAAACAACATATCAAAAAAATTAAGTCTGTCAAAACTCAGTTTTAAGAAATGAATTAATTTCTCAGGTGATACAGCGCATCTCACTATTTTTGACAAAAATA ATAATGTTAAAAAGTAAACTATCTTTGATACCAATGTAATTTAGTAAAAATGTCACAAATAGTTGTATAAACCGTATTCTAAAAACAAGTAACTGCTGTCAAAACTG AATTCAAAAA
<b>TbrSat139</b>	GGATTTTTTCTACAATCTTACTCAATGACAATATATTTGAGATAGTAAATTAACAAGAGTCTCAATAAAAACTCTCTGACACACATCTGATGCAAGCTGAGTGT CGGCTGCACCATTTCTGATTTCTGCAAGAAAAATTTAGGATGACTATAAATTTACTCGTACCAAGAAATTAATAATGCAAACTTTTGAATTTTAAAAACAATGT AATTTTTTTTTTGTAGCACAAAAAATTCAGCAAGGTTTCAATAAAGCAAAAAACTGATTTTTTCTATAGTGAAGGTTAATAACAAAAATTTTTTCTAAAAATAAG TTTTATCATCAAT
<b>TbrSat140</b>	CTATGACGCATTTGACAGTTTTTTGACATACAATTTTAGACAGTTGTCAAATCTTGAACATTTACTAATTAACAAGAAAAAAGATGTTTTTGTAGTCTTGAACCG ATAATTTGTTTTGACACCTCTTTGACAACGCAAAATATAAATGCAAAATCTGTAAAGTTTACGCCAATTAACAAGTGAAGAATATTTTTAGGTACAGCATTAA CCATTAACCTTTAGTAAACATTAAAAAA
<b>TbrSat141</b>	GAGTCAATAAATCTTACGCAAGTTGAGTCTTTTAAAGATTTTGGCAGTAACTTCAAAATTTGCTCAATCAGAACAATAAATTTTGTGATTTTGTAGTCAAGCTGT GATGGTAGAAAACTAGCTTAAATGCAACAATAAAGTATTAGTCAATTTTTAAAAAATTCGCTTACATATTTGCTTATA



<b>TbrSat142</b>	GTTAATATTAATGTTTTCTATGTAAACAATCTGGGAAAACAAATTTTAAAGAAAATACGTTTTCTAATCTTTAGGAATTAAGAGCAAATTTATGAGACGCTGATACCACCCGACAACAATTCATTTTTCTTATAATATGTTAAAGAAACGACAGCAAAATTTATTTTTGTACAGAGAATTAACGATAATTAACAAACCCGAAAATTCATTAAGCTCCTAAAATTTGGTTTTGAAGATATCGCAAAGAATTCAGAGCGCAGCAGCAAAAATTAACAACTCGCTCGTAGCGTTTGAATAAATGCGATTTTTCTCGGAAAATTTTTAGTGGAAATTTAGTAAATTTTCGTACACTTATGTAATAAAGCTGATTTCAAATATGGCACATTTATTTATCTGAGGTTATAGTTCAAAAATATCGCTAAAAGTTTAGAGAAAGCGCTCATAAGGTTAAAGTATTAGCCGTTGCATCAATACTAAATTAACAAAATTTGCTTCAAATTTGCTTTTTCTAAAGCAAAAATAAAGCTGTTCAAAAATGCGCTTAGAATTTATCTATGACTTCTAGTTTTTAAATACCTAAAAATAACGACTTACTCACAAAAAAATGTGGACAGACCATAATGTTCTTATAATTTAAACAATACCAACCAAAAAA
<b>TbrSat143</b>	GGTCAAATAAAGGAATAAATACACAAGTCTAAAAATAGTCATATTTTACATATTTTCGTGTGCAAAAATACTAAAAATTTTTCTCCCTGTTTTAAAAATAAAAAACAAGGAGAAITTTAAAAATTTGTCAAAAATATA
<b>TbrSat144</b>	GATTTTTGGTAATGACGAAAATTTGTCTAAAGTGAATAATTTAGTACAAAAAAATTAACCTACACTTCAACAAAAGTAAAAATGTAATAATAGATACATCTTATGCAAAAATTAATAAGAAATCTAAATCTGTAAGGTTTTAAAGCTACAGATACTA
<b>TbrSat145</b>	AAGCATGAGGCAATTTTTTTTTGTCAAAAATTTGCAATTTGGCGATAGTATATCTCTAAAAACAACGATATCGTAGTTGAATTTTCAGCCAATATTAATTTCCAGTACTAGTACATTTCTGCTAAGACGAAATCACACGATCAATTAATTTGACGGAACTATGAAAAATTTGTTTTAAATTTAATTTTCTAAAAATAATGAACTTTGACGAGCATAACTTCAAAAACAGTCAGAAAATGATAGTTTTTGCATTTTCGGATTTCTGTCTAATTTTACGTCGAGAAAAGTGTGTTTTGAAATTTTTCTGTGCGTTTTATGGAAAATATACTTA
<b>TbrSat146</b>	TGTTGATAATTCAAAAAAATTTGTGAACCTGTATCAAGTAAAAAGATTGATTAACCAATTAAGGCGAGATACAACAAGATTTGGTGAAAGCAGAAAACAAGAAAATTTTTAGAAATCATATTTTTCCGAAAAATAGAACAATTTTTATGGATGAA
<b>TbrSat147</b>	GAATTTTTTAGGAAAAAATCTAGAAATTTTACGATTTCTAGAGATTCGAGTAGGATTTAGCACACTTCTAGAAAAGTGTAAAAAGTTTTCTTTGGCTCTAGAAATCAAAAATAAGCTGTAGATAGATTTTTCTAGCCACTCTAAGTCCAGAAAAATTTCCGGAAGATACTTTCTAAGTTCTA
<b>TbrSat148</b>	ATAGACGATTTGTTTTCAAAAAACAACGCAAAAGTAGAAAAATGTAATAAAGTTCATCGGATACTAACACTTTGTACAATTTGTGCGTATTTCAATAAATGACAGTCAAGGTCAGGTCATACTTGTGGACCTAAATTTAAATAAAGGACCCTGATTTCCGTGAGATATGACACAGTAATACAGAGTTAATATCTGATGAATGC
<b>TbrSat149</b>	GGAGTTTTCTAAGACTATAGTTTTTCAAAAAACAATAAAGCTAAAGAATTGGATTGCAGGCAAGCATCTGATCTATCAACGAACAAGCACAGATTTCACTTTGCAAAAGATAATCTACTTTAGAGATCGTCTAGAAATCAAAAAGTTCGGGTTGAACAATTTGTTTTAAATTTTAACTCCGGAAGAGTGCCAGCTGAAGTTTTGCGTCAAAATTTCAAAAATTTGTGACACAAGTGTATGAAAAATCCACACACTTATAGAAAAATCTCAAGGAAAATGACGCCATTGACT
<b>TbrSat150</b>	GAGATTTTTAACTGGCAATTTACTCGTGAACCTAAGGGTGTCAAAAATTTGGTAAGAAGTGTCAAAAATTTGCAAAAATTTAATGTTTTAACTGGTATAAAAAATTTGCAATACGTTAACTGGGGTGTGCG
<b>TbrSat151</b>	TTGTTGCTCTAGATACCACTTTCAAAAATATGGTCAATTTTTGAGTATCTGCCTCAGAACAAGCAAACTTTCCATTTTATTAACAACCGTTGAATGATTATTAAGTAAAGATTTACAATTTATTGGTT
<b>TbrSat152</b>	AACTGAAAAATAATTTGGTAGCAATTTTCAAGTCCACTGACGCTTTGATTCTAAGATGGCGCTTTGAAAATAATGATTAGAATTTGTAATTTGGAGCGCCGAAAAATCCCCAGTTTTTGTAAAAACGGTACTGCATCTATAAAAATTAGTGATTTATTGGGAAAAAGTTTTAAAGTATACTAGAATCAAAATAAAAAACGGTACACACCGAATTTGTTTTATGATTTCTTAGTTGAATTTAACTTAGTGGAAAATATCGTCA
<b>TbrSat153</b>	TTATGGATAACACTATGGAATTTTTATTATCTATTTCCAGAAGTACTTTATATCCATCTTTAGCCTTGGCTATAATGAATCAAAAGTACTACCTCTTTATTGCTCTATAATTTGATGACTTGACCCTATAATTTCTAGAAGCGTTACTAAGATAACT
<b>TbrSat154</b>	AGAAAAAGGCTAACATTTTTGGTATGATATTTCTACAATTTTCGACTTCTAATAATTTGCTTTGGAATTTAAAGTATTTTTTAAAGCTGATGTTAAGTTAAAAACTTTAAAAATTTGGAGAAATTTCAAAATGTCAAAAAGTTTTCAAGTTCAGAAAATGATGTAAGTTTCTAGAAATTTAAAGATTTTTATAATTTAGAGCTTTCTGGAAACACATGGAACTTCCGGAATAAAAAATTTATCTTTTTTAAACAACAATCTTAAACAATTTGTTGAGAAATTTAGGGGCTTTTGTAAAGTTTATTTTTAGAAITTT
<b>TbrSat155</b>	ATTTATAATGCAACTACCTATCAAAATTTGAATTAAGTAACTTCGCAACATAGCCGGGGTCTTACGTCCAAATAAATAATTTACTGTTATTAATAGCATGAAATTAATAACCAAAACTTTGTTGAAATTAATTAAGTAACTTTCT
<b>TbrSat156</b>	TCTTGAGTTTTAAACAAGAAAATCGGTATTTTAAAGATTCAAAAATTTGATTGTTTTGACAGTTTTGATTATGTTTCAATAACGTTTTAGTTAAGCTGGATTAAAAAATAAGTATTTTTGCATTGAATAATTTGTTAGTTTTGTCAAAATTTGACTGTGTAA
<b>TbrSat157</b>	CAGAAAACCTGAAAAATTTTACAATACTTAATTTTTATGTTGAAGAACAAAAAGTAGTAACCTCAGGCCCTTGGAACTCCGTTTTTAAAAATATATAAAAAATTTTTGAAAGCTTTAGAAAACGATATAAAGCCATATGAACTTTTTGAAGCTTCTAGGAATGATATAGAGCCTTACCTTAAATCAAGATCCTAGAAAAACCTTAGGAAATTTTCATAGAAATCTAGAAATCTTAAAACTCTGGAGAACGCTCAAAATTTTGCAGAACTGTTAAAAATAAAAAAATTTGGTGAATA
<b>TbrSat158</b>	AAAGTGTGTCAACAAAATAAAGTCCCAATATAAAACATTTTCAAAAATGGGTAACAATAACAATATTTGTCCATTGAGACTGACGGATCAAATAAGCCATAAATACATAAAGAGCTTTCTTTAGTGTGTTAGTTGTATAGATGATGACTGACTCTACCAACATCACCAGCATAAAAAACCTTCTGGGATGATTGTTAAAAAATACAGTCAATTAATAACTCAATTTATAAGTAATTTATAGTTACCAACGATAAATATCACACGATATGATATCTATCACGGTTGGCAACGCTGCGGTGAGAGGTTAGTTGAT
<b>TbrSat159</b>	GGATTTCTGATATTTCTCAAAATTTCTCTATTTCTAATAAATTAACAATAATTTCTTAAITTTTTACCTTTTCTGAAACAGTTTACAGATTTCTCTAATTTCTACTCAAAATTTCTGAAATTTCTGATTTATTCTCAAAATTTCCGTAATTTCTAATAAATTTCTAGTACTTTTGTAAATGCTACATTTTTCTTACCAAAAATTTCCA
<b>TbrSat160</b>	AGGATTTCTAGAGCAAGAATTTGACTGAAAAATGCGTTTTGTAATGAAAAATTAGGGTCTTACCGTGTAGGGAAATTTATAACCATTTTTCTGTTTTGGTAAAGATGACTGAAGTTGGCGCTTTGTAAGTATACCAACCAAAATTTTATCAATGTTGGAAAAAATTAAGAAAAAACT
<b>TbrSat161</b>	AGCAATTTCTGTATCAAAAATGTCAATTAATCTGACTACCATCTAGGTCTGTTTTAAAGTAAAAATAGATTAAGATCGGAAACGTAACACACACAATAAGCCAATAAATCGAAATCAGGTCCAGTCAATAAATAAATACTCATCTCAATCTTACGTATAATAAATAACTCGCCTAAATCTATGCTACTTATTTTTATCTATTTATTTTTTAAACAGCAAAAATAAATGGTAACTCATCTATATCT
<b>TbrSat162</b>	CCAAATTTGAAACGTTGCGTATTCTAATTTGGCAGATAAAATCAAAAATCTTTGTTTTAACACACTAGCGTAGTATAAACGTCAAAAACTATCTAATACTTAACTTTTATGCAACAATTTGAAAAAACAATCATTTTTGAAAGGTAATGAGTTCAATTGACAATTAATAACCCCTGATTTAGTAAAAACAGAAAAACCAAAATTTATCTTTTCCAAACATAAACAGTTTTAA
<b>TbrSat163</b>	CAATGTAATAATAACTGTTTACTAGATTTTTATAACTTATTTTTAAATGATATTGAGACACTGTAATAATACATAAAGAAATTTAAGTAAAAATTTAATAGGCTTTTAAATTTAAATTTAATAAAAAATAACAATTTACAATTTAGTAATAATGTAATAAATTTCTAATAATTTCTGAGTTTTAATGTAATAATTTACAGAAATGTTAAAAATGTTAAAAATCAAAATAAATACTTACTCAACTAAGAGATTTTTAAAAATAACCTGT
<b>TbrSat164</b>	GTTCAACAAGCTCAATATCCCACTGCTGATGATAATTACATTAATACATTTTTCTAGCCTTTATGTGTCACCACAACAGCTTCTCATTTACGGTTTCGATTGAAAAATGCAATGAAAAAGAGTGGGAAATTTGCACACATGCTCAATTTTCGAAAAAGTATTCCAATTTTGACAAAAT
<b>TbrSat165</b>	TTTAAAAACGTCATAGTTACAATTTTTATGTAATTTTACTTAATGTAATTTAAATTTATCATCTTTGTAATTTTCAAACTAAAAAGCTTATTTTACCTTAATTTAATCTCAGTATTTTTGAAAGATTTTTAAATAAGCAAAATTAATAGTGAATTTTTACACGAGGAGATGTAATTTTACAATTTTCAATATGTCGCTATAAAATTTAATAATTAATAAATGGTACACAGTTTTTCAATTTACTTAACAGCTGTGTAACCTTTAAATGTAAT
<b>TbrSat166</b>	CTTGAGATCAAAAATTTGGTATTCCCAAGAAATGCTGCTTATTTGTAATGTTGGTCTTTAGGAAAGAGTCAAGTTGAAGAATTTGGAGGGTATGGAAGAAGTCAATTTGAAAAAAGCTGGTATCAAACTGGACGAAAGACTTCTACAATGAAACCGAGAAAAAATATGCTAGTTGGTGAAGAAGGTTGCAAAAATCATCAATCTTTCTGTTCTTTCCGGTTGTATGACTTCGAAGGAAAAATCACCATAGATGGTGTGATCAAAAAGTGTCAATTTGCATTT

**Supplementary table 2.** The list of *Tribolium freemani* satellite DNAs that share partial similarity with transposable elements recorded in Repbase database (Kohany et al. 2006). DNA transposons are marked in red.

satDNA	RepBase partial hits	satDNA	RepBase partial hits	satDNA	RepBase partial hits
TfrSat02	Sola1-2_BM, DNA8-95_AP, Transib-N1_CQ, Gypsy-6_TrVa-I, hAT-104_HM	TfrSat50	LIN10_SM, hAT-15_Can	TfrSat86	Ginger1-1_AP
TfrSat11	Gypsy-127_ZM-I	TfrSat51	Gypsy-33_DEI-I	TfrSat87	Gypsy-35_PSt-I
TfrSat15	Rehavkus-1_TC	TfrSat52	MuDR-34N5_MT	TfrSat88	Sola1-5_LMi
TfrSat17	IS3EU-1_TC	TfrSat53	CR1-48_HM	TfrSat90	hAT-6_RPr, KolobokE-1_TC
TfrSat18	Polinton-6_HM	TfrSat55	HARB-N17_BOI	TfrSat92	Gypsy-19_MiUn-I
TfrSat21	MDR2	TfrSat56	Mariner-3_BM	TfrSat93	Gypsy1- SM_LTR, hAT-N5B_EL
TfrSat22	hAT-N26_Aly, rn_364-201_LTR	TfrSat57	MuDR-N8_RSa	TfrSat94	DNA-1_CQ
TfrSat23	Rehavkus-3_TC	TfrSat58	Mariner-85_HM	TfrSat97	Chapaev3-3_PM
TfrSat26	MuDR-2N1_CorFlu, DNA6-13_Cgi	TfrSat60	LIN13_SM	TfrSat98	DNAV-1f_LVa
TfrSat28	hAT-N77_OS	TfrSat61	LIN25_SM	TfrSat101	BEL-20_SiCu-I, Medea
TfrSat29	ERV1-4_ThAm-I	TfrSat62	hAT-35_HM, Mariner-25_OT	TfrSat102	Copia-168_Aly-I
TfrSat30	Helitron-N12D_LMi	TfrSat63	Medea	TfrSat103	DNA-1_CQ, P-19_HM
TfrSat31	Helitron-N68_CGi	TfrSat64	Transib-7_DAn	TfrSat104	MuDR-3_Mad, GIZMO2_EI
TfrSat32	Tlr1	TfrSat65	Chapaev3-2_HR	TfrSat105	IS3EU-1_TC
TfrSat33	Caulimovirus-7_ArHy, Jockey-7_Dta, Athena-YJ_As	TfrSat66	hAT-N23_Aly	TfrSat106	hAT-N12_RPr
TfrSat34	MuDR-34N6_MT	TfrSat68	Gypsy-12_SpEx- LTR	TfrSat110	Harbinger-6_CGi
TfrSat39	hATx-3_SM	TfrSat70	Copia-9_OS-I	TfrSat111	L1-80_ACar
TfrSat43	hAT-131_HM	TfrSat72	DNA2-3_TCa	TfrSat112	Gypsy-55_TrVa-I
TfrSat44	hAT-163_HM, Gypsy-696_AA-LTR	TfrSat73	MuDR-6_TV, piggyBac-1_Cgi, hATm-1_HM	TfrSat113	KolobokP- 7_MoPh
TfrSat45	GYPSY2-LTR_MT	TfrSat74	Copia-23_NVi-I, RTEX-61_Cgi	TfrSat117	CryptonH- 10_HM, KolobokH-1A_Rir
TfrSat47	MuDR-N16_Rsa, Helitron-N21B_Aly	TfrSat76	MuDR-1_Cba	TfrSat118	Gypsy-131_DTa-I
TfrSat48	Gypsy-34_Mad-I, CER15-1-I_CE	TfrSat79	DNA-11_HM, UroGra-5.2312, Gypsy-13_CySi- LTR	TfrSat119	TRAS-5_Tur, Helitron-1_DK
TfrSat49	hAT-15_DTa	TfrSat82	KolobokE- 1_TenNot	TfrSat122	Gypsy-6_EPa-I



**Supplementary table 3.** The list of *Tribolium madens* satellite DNAs that share partial similarity with transposable elements recorded in Repbase database (Kohany et al. 2006). DNA transposons are marked in red.

satDNA	RepBase partial hits	satDNA	RepBase partial hits	satDNA	RepBase partial hits
TmaSat01	EnSpm-N9B_CGi	TmaSat41	DNA-1_CQ	TmaSat78	HARB-1_Stu, Helitron-10_CGi
TmaSat02	MuDR-11_MT, EnSpm-15_DR	TmaSat42	Gypsy-22_DTa-I, MuDR-N45_MT	TmaSat79	DNA-30_LVa, Gypsy-19_MiUn-I
TmaSat03	Kolobok-N2_DTa, EnSpm-14N1_DR, Tx1-7_CorFlu, hAT-N62_RSa, Gypsy-5N_PPa-LTR	TmaSat43	SCL18_TatV	TmaSat80	TE-X-11_DR, Medea, Gypsy-53_AllFus-I
TmaSat04	Sat-2_STu	TmaSat44	Gypsy-16_LSal-I, MuDR-13_CGi	TmaSat81	DNA-1_CQ
TmaSat06	ISL2EU-N3_BTa	TmaSat45	HOBO	TmaSat82	Gypsy-48_AnFu-I, EnSpm-2_GM, Mariner-N1_DGr
TmaSat07	Harbinger-3_TV, Gypsy-3_PPP-I	TmaSat46	MuDR-12_GM	TmaSat83	EnSpm-4N1_HM
TmaSat08	REP4_XT	TmaSat47	LINE-1_AA	TmaSat84	Gypsy-28_Cas-LTR, Gypsy-59_TrVa-I
TmaSat10	BEL-35_LiLo-I, Gypsy-7_BIJa-I	TmaSat51	Helitron-2e_Ipa	TmaSat85	DNA-1_CQ, Mariner-7_LCh
TmaSat15	TA11	TmaSat52	Copia-5_CoGI-LTR	TmaSat86	Gypsy-7_BIJa-I
TmaSat16	Fanzor1-1_HemFu, Helitron-N10_LMi	TmaSat54	Gypsy-28_DMo-I	TmaSat88	EnSpm-1_Gar, P-14_HM, Kolobok-N1_DWil, hAT-125_HM
TmaSat18	EnSpm1_SB	TmaSat55	MuDR-N45_MT	TmaSat89	Copia-13_TC-I, Transib-7_HM
TmaSat21	Copia-1_XylGra-I, Helitron-17_ArHy	TmaSat56	MuDR-N3_NS, TABARE	TmaSat90	Mariner-52_HSal
TmaSat22	Helitron3_PPa	TmaSat57	Copia-53_MT-I	TmaSat91	HELITRONY1E, EnSpm-26_HM, EnSpm-N1_DEI
TmaSat23	Gypsy-26_CGi-I, hAT-2_SD	TmaSat61	Copia-22_MNI	TmaSat93	EnSpm-5_ArHy, MuDR-34N2_MT, Helitron-N2_AT
TmaSat24	DNA-14_LSal, Penelope-9_SiOr	TmaSat62	hAT-8N1_ATr	TmaSat95	Mariner-2_DSuz
TmaSat25	Naiad_Ptep	TmaSat63	Medea	TmaSat96	hAT-N33_Bol, Utopia-1_DYak
TmaSat26	Mariner-71_MeMe, ERV2_MD_I, CR1-12_DTa	TmaSat67	MuDR-34N4_MT	TmaSat99	Gypsy-17_PMon-LTR
TmaSat28	Gypsy-14_ODI,	TmaSat68	DNA-11N_SBi	TmaSat100	hATx-20_SM

	Sola2-1_DTa				
<b>TmaSat31</b>	MuDR-N1_BOI	<b>TmaSat69</b>	Helitron-4C_LSal	<b>TmaSat101</b>	EnSpm-3_HM, piggyBac-14_SM, Fanzor1-2_LepBou, Gypsy-413_ZM-LTR
<b>TmaSat32</b>	BEL-5_DSer-I, hATx-5_HM	<b>TmaSat70</b>	Copia-24_GR-I, L1-37_AAe	<b>TmaSat102</b>	KolobokP-2_PoSt
<b>TmaSat33</b>	Gypsy-131_DTa-I	<b>TmaSat72</b>	MuDR-N16_MT, P-1N1_DAlb, Medea	<b>TmaSat106</b>	Gypsy-14_Cas-LTR, Gypsy-42_CAn-I
<b>TmaSat34</b>	Tx1-17_CGi, Tx1-6_CGi	<b>TmaSat73</b>	hAT-89_SM	<b>TmaSat108</b>	MuDR-N7_DaCa
<b>TmaSat35</b>	Mutsu-4_PaRa	<b>TmaSat74</b>	Polinton2_SM, DasNov-4.624, MuDR-6_GM	<b>TmaSat109</b>	KolobokE-4_CanRus, EnSpm-4_RSa
<b>TmaSat36</b>	hAT-4_RC	<b>TmaSat75</b>	ISL2EU-N3_BTa	<b>TmaSat111</b>	Gypsy-6_MeUr-LTR
<b>TmaSat37</b>	Gypsy-182_AA-I, Gypsy-39_TeGr-I	<b>TmaSat76</b>	Loki-1_FuHe-I	<b>TmaSat116</b>	Gypsy-59_GR-I
<b>TmaSat38</b>	Caulimovirus-5_JC	<b>TmaSat77</b>	Medea, ISL2EU-5_BTa	<b>TmaSat119</b>	DNA-N24_DR
<b>TmaSat40</b>	Zator-2_HM				

**Supplementary table 4.** The list of *Tribolium confusum* satellite DNAs that share partial similarity with transposable elements recorded in Repbase database (Kohany et al. 2006). DNA transposons are marked in red.

satDNA	RepBase partial hits	satDNA	RepBase partial hits	satDNA	RepBase partial hits
TcoSat03	EnSpm-20_Aly	TcoSat39	Copia-4_PirFin-I	TcoSat71	Gypsy-16_DEI-I
TcoSat04	hAT-N2_AN	TcoSat41	HARB-N21_RSa	TcoSat75	Daphne-25_HM, HARB-N23_Aly
TcoSat08	BURRO3_LTR	TcoSat42	Gypsy-16_DBP-I	TcoSat77	Gypsy-62B_DWil-I
TcoSat10	hAT-N1_DaCa	TcoSat43	Gypsy-8_DPer-LTR	TcoSat80	DNA-1_CQ
TcoSat11	SINE3-1_SpEx, Gypsy-55_LMi-I, DNA8-28_DR, SINE3-1_SpEx	TcoSat44	Helitron-24_OS	TcoSat81	MuDR-N16_MT, Gypsy-34_PirFin-LTR
TcoSat12	RTEX-1_OwFu, L1-54_RSa	TcoSat45	Gypsy-95_Aly-I	TcoSat83	Gypsy-19_GAr-LTR
TcoSat13	REP-2_PBa	TcoSat47	hAT-14_CoLJ	TcoSat85	DNA-1_CQ
TcoSat16	hATm-15_HM	TcoSat48	Gypsy-222_AA-I	TcoSat87	hAT-3_PBa
TcoSat17	MINISAT2_CB	TcoSat49	Transib-1_Sln	TcoSat89	Helitron-N3_PPa
TcoSat19	Mariner-21_LCh	TcoSat50	EnSpm-16B_TAe, Copia-53_MLP-I	TcoSat91	HETA_DSi
TcoSat21	MINISAT2_CB	TcoSat51	Gypsy-15N_PPa-LTR	TcoSat92	KolobokE-3_LimLun
TcoSat22	hATm-15_HM	TcoSat54	Daphne-105_HM, P-1N_HM, Gypsy-45_LVa-I, DIRS-5_SiOr	TcoSat93	piggyBac-17_SM
TcoSat24	Harbinger-1_GAr	TcoSat57	LTR145_MD, Helitron-20_CGi, Gypsy-7_SpEx-I, Mariner-29_HM	TcoSat97	Gypsy-10_PAb-I
TcoSat27	I-1_BTa	TcoSat59	Mariner-8_SS	TcoSat98	Gypsy-25_CAN-I, Gypsy-7_JC-I
TcoSat29	Ginger2-1_CySi	TcoSat61	MUDR5A_CB	TcoSat101	Dada-U6_EtSp
TcoSat30	Mariner-34C_OT, hATm-52_HM, CRE-5_FoCa, KolobokD-8_SaGl	TcoSat65	Gypsy-124N_SBi-LTR	TcoSat104	MuDR-5_SeTo
TcoSat33	HARB-N8_DRot	TcoSat66	Gypsy-18_AnSt-I	TcoSat106	DNA7-N1_DR
TcoSat34	MuDR-2_Aly	TcoSat67	Helitron-N29_Aly	TcoSat107	Mariner-N8B_Stu, Gypsy-38_LyDi-I
TcoSat36	Gypsy-35_SM-LTR	TcoSat68	Gypsy-9_PirFin-I	TcoSat108	TelKA1_Av_I
TcoSat38	MuDR-N9_RSa, SAT-2_DAZt	TcoSat69	Gypsy-68_LMi-I		

**Supplementary table 5.** The list of *Tribolium brevicornis* satellite DNAs that share partial similarity with transposable elements recorded in Repbase database (Kohany et al. 2006). DNA transposons are marked in red.

satDNA	RepBase partial hits	satDNA	RepBase partial hits	satDNA	RepBase partial hits
TbrSat01	Gypsy-83_CGi-I, Gypsy-16_ChVe-I, Academ-7_LMi, Guadalupe-1, Gypsy-28_DMel-I, Mariner-N5B_DR, Gypsy-17_MeUr-I, Transib-1_PPo	TbrSat58	Daphne-78_HMa	TbrSat117	Sola1-1_HM
TbrSat03	Daphne-45_HM	TbrSat61	Copia-2_PirFin-I hATm-6_HRo, L1-37_ACar	TbrSat118	MuDR-N19_RSa
TbrSat09 <sup>B</sup>	EnSpm-3_MT	TbrSat62	Kolobok-2_AP, VANDAL8, DNA2-1_CB	TbrSat119	Tad1-74_PoXa
TbrSat10	Rehavkus-1_NVi	TbrSat63	Helitron-3_ArHy	TbrSat120	Gypsy-8_LH-I
TbrSat12	hAT-97_HM	TbrSat66	EnSpm-31N1_OS	TbrSat121	MuDR-25_ArHy, piggyBac-1_PMon
TbrSat13	Polinton-6_HM	TbrSat67	hAT-8_HRo	TbrSat122	hAT-11_HM
TbrSat14	Sola3-3_BF	TbrSat68	hAT-2_ILMP	TbrSat123	Helitron-15_ArHy
TbrSat15	CryptonV-N3_DR	TbrSat70	Ogre-PT1_LTR, ERV1-3_SaFa-I	TbrSat124	DNA-1_CQ
TbrSat16	Gypsy-13_Hmel_I	TbrSat72	BEL-9_EmpOnu-I, KolobokD- 8_CorFlu	TbrSat125	EnSpm-N21_DTa, MuDR-N1_ALy, Academ-N3_CorFlu
TbrSat17	Mariner-57_OT	TbrSat73	Zisupton-3_DR	TbrSat126	Osa_Abermu, R4-1_TCa
TbrSat18	MEGY-I_MT, Gypsy-5_LSa-I, Caulimovirus- 15_ATr, DNA3-6_STu	TbrSat75	DNA-1_CQ	TbrSat128	Mariner-27_HM
TbrSat20	Helitron-N10_LMi	TbrSat76	LIN7B_SM, MuDR-N16_MT	TbrSat129	PROTOP
TbrSat22	Transib-10_HM	TbrSat77	Mariner-15_CFI	TbrSat130	Gypsy-6_RO-I
TbrSat23	Itch-19_DeSi	TbrSat78	MuDR-N16_RSa	TbrSat131	BEL-23_ColCro-I, DNA-1_CQ
TbrSat25	Gypsy-106_OS-I	TbrSat79	Ginger2-4_OT	TbrSat133	Helitron-2_EPa
TbrSat27	Tad1-26_BG	TbrSat80	DNA2-2_BTa	TbrSat134	Zator-5_Rlr
TbrSat28	KolobokE-3_AP	TbrSat81	HELITRON7_CB	TbrSat135	Fanzor1-2_LepBou, MuDR-3_SBi
TbrSat29	RAS2_MT, Merlin-4N3_OT	TbrSat82	DNA-1_CQ	TbrSat137	MuDR3_SM, DNA-4_PBa
TbrSat30	MuDR-8_Cas,	TbrSat86	hAT-47_SM	TbrSat138	EnSpm-N42_DR

	Gypsy-9_PirFin-LTR				
TbrSat32	hAT-N16_CiSi	TbrSat87	Gypsy-443_AA-I, hAT-2_GM, Copia-90_ST-I	TbrSat139	EnSpm-7_Stu, Ginger2-5_OT
TbrSat33	EnSpm-5_VV, Mariner-24_LSal, Mariner-51_OT	TbrSat88	RTEX-75_CorFlu, EnSpm-2_TD	TbrSat140	MuDR-1_LMi, Copia-4_DEI-I
TbrSat34	Gypsy-133_GM-LTR	TbrSat89	MuDR-14N1_OS, MuDR-8_VV	TbrSat142	hAT-N3_Drot, KolobokD-3_CySi, Rehavirus-1_DA
TbrSat35	PinfV, EnSpm-9_MT	TbrSat90	Harbinger- 24_CorFlu, Chapaev-14_HM	TbrSat143	ATMUN1
TbrSat41	DNA8-98_AP	TbrSat95	Sola2-4_NVi, hAT-N19_DRot	TbrSat146	GLT_SM, MuDR-N17_RSa
TbrSat42	AcademH-15_CGi	TbrSat97	Polinton-10_SM, hAT-N17_RPr, DNA-4_RPr, DNA2-10_CGi	TbrSat147	EnSpm-16_RSa
TbrSat43	KolobokP-1_MoPh	TbrSat98	hAT-39_DWiI, ISL2EU-33_CGi	TbrSat148	TART-1_DWi, KolobokE-3_MyEd
TbrSat44	DNA-1_CQ	TbrSat99	Harbinger1_PTr, AcademH-9_CVi	TbrSat150	HARB-N10_OES
TbrSat45	DNA-1_CQ	TbrSat100	hAT-14N1_ZM, Tx1-60_DR, hAT-3_TV, RTEX-33_CGi	TbrSat151	Gypsy4-SM_I
TbrSat48	Gypsy-135_CGi-I	TbrSat105	EnSpm-2_Cia, MuDR-6_VV	TbrSat154	Gypsy-34_PirFin- LTR
TbrSat49	Helitron-12_ZM, Sola2-11_HMa	TbrSat107	KolobokH-3_Rlr, Mariner2_PPpa	TbrSat157	DNA-1_CQ
TbrSat50	Gypsy-69_CySi-I	TbrSat108	P-10_LSal, piggyBac-4_Ilyo	TbrSat158	Gypsy-64_OL-LTR, Gypsy-24_RC-I, DNA8-2_DR
TbrSat51	Daphne-25_SPur, BEL-13_CarQue- LTR	TbrSat109	Gypsy-67_CAnI	TbrSat159	DNA-1_CQ
TbrSat52	LR9A	TbrSat110	CRE-27_CydSpl	TbrSat161	Copia-147_MT-LTR
TbrSat53	Copia-3_Mac-LTR	TbrSat111	Gypsy-6_RO-LTR	TbrSat162	Tx1-53_DR
TbrSat54	KolobokE-1_ZePy	TbrSat112	L2-22_CTe, MuDR-N7_AT	TbrSat164	L1-119_DR
TbrSat55	DNA9-N1_DR	TbrSat114	Chapaev3-1_OL	TbrSat165	Ginger2-1_Rlr
TbrSat56	L1-19_DR	TbrSat115	Kolobok-N1b_TV, Gypsy-3_DiPu-I, MuDR-3_GAr	TbrSat166	Gypsy-1_TuIn-I
TbrSat57	PARISa-2_DW	TbrSat116	Gypsy-7_DBi-I, Helitron-2_EPa		

## 9.1 List of abbreviations

<b>PacBio HiFi</b>	Pacific Biosciences High Fidelity sequencing
<b>BLAST</b>	Basic Local Alignment Search Tool
<b>bp</b>	base pairs
<b>CAFs</b>	cancer-associated fibroblasts
<b>Cy3</b>	Cyanine3 fluorophore
<b>DAPI</b>	4',6-diamidino-2-phenylindole dihydrochloride
<b>eccDNA</b>	extrachromosomal circular DNA
<b>EDTA</b>	Ethylenediaminetetraacetic Acid
<b>ENCODE</b>	The Encyclopedia of DNA Elements
<b>ExoV</b>	exonuclease V
<b>FISH</b>	fluorescence <i>in situ</i> hybridization
<b>FITC</b>	fluorescein isothiocyanate
<b>Gb</b>	gigabase
<b>HMW DNA</b>	high molecular weight DNA
<b>HSF1</b>	Heat Shock Factor 1
<b>HOR</b>	Higher Order Repeat
<b>IPTG</b>	isopropyl $\beta$ - d-1-thiogalactopyranoside
<b>kb</b>	kilobase
<b>LB</b>	Luria Broth
<b>lncRNA</b>	long non-coding RNA
<b>LTR</b>	long terminal repeat
<b>Mb</b>	megabase
<b>MITE</b>	miniature inverted repeat transposable element
<b>MNase</b>	micrococcal nuclease
<b>Mya</b>	million years ago
<b>NGS</b>	Next Generation Sequencing
<b>ONT</b>	Oxford Nanopore Technologies
<b>PCA</b>	principal component analysis
<b>PBS</b>	Phosphate Buffered Saline
<b>PCR</b>	polymerase chain reaction
<b>PMSF</b>	phenylmethylsulfonyl fluoride
<b>RNAi</b>	RNA interference
<b>RT</b>	room temperature
<b>satDNA</b>	satellite DNA
<b>SDS</b>	Sodium Dodecyl Sulfate
<b>SINE</b>	short interspersed elements
<b>SOC</b>	Super Optimal Broth with Catabolite Repression
<b>SSC</b>	Saline Sodium Citrate
<b>STR</b>	short tandem repeats

<b>TAE</b>	Tris-Acetate-EDTA
<b>TAREAN</b>	Tandem Repeat Analyzer
<b>TBE</b>	Tris-Borate-EDTA
<b>TE</b>	transposable element
<b>TR</b>	tandem repeats
<b>TRF</b>	Tandem Repeats Finder
<b>UTR</b>	untranslated region
<b>X-gal</b>	5-Bromo-4-Chloro-3-Indolyl $\beta$ -D-Galactopyranoside
<b>2D</b>	two-dimensional

## 10. CURRICULUM VITAE AND PUBLICATION LIST

Ocjena rada  
u tisku



## Curriculum vitae

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**Name: Damira Veseljak**

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## Education

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- 2020 – J. J. Strossmayer University of Osijek, University of Dubrovnik, Ruđer Bošković Institute, Zagreb - University Postgraduate Interdisciplinary Doctoral Study, Molecular Biosciences
- 2017 – 2019 University of Zagreb, Faculty of Science, Zagreb, Croatia, M.S. studies in Molecular Biology, master thesis "Characterization of satellite DNAs of the flour beetle *Tribolium freemani* Hinton"
- 2018 – 2019 University of Girona, Faculty of Science, Girona, Spain, Erasmus+ mobility, fall semester
- 2014 – 2017 University of Zagreb, Faculty of Science, Zagreb, Croatia, B.S. studies in Molecular Biology
- 2010 – 2014 Prva gimnazija Varaždin, bilingual programme

## Professional experience and training

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- 2019 - Ruđer Bošković Institute, Zagreb, Croatia, research assistant and PhD student at Laboratory of non-coding DNA
- 2018 – 2019 University of Girona, Faculty of Science, Girona, Spain, laboratory skill training at Laboratory of Gastrointestinal Diseases
- 2018 University of Zagreb, Faculty of Science, Zagreb, Croatia, laboratory skill training at Division of Microbiology
- 2017 Ruđer Bošković Institute, Zagreb, Croatia, laboratory skill training at Laboratory for Molecular and Cellular Biology
- 2017 – 2018 University of Zagreb, Faculty of Science, Zagreb, Croatia, Student teaching assistant in Molecular Genetics
- 2016 – 2017 University of Zagreb, Faculty of Science, Zagreb, Croatia, Student teaching assistant in Plant Physiology
- 2015 – 2016 University of Zagreb, Faculty of Science, Zagreb, Croatia, Student teaching assistant in Cell Biology

## Publications

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Volarić, M.; Despot-Slade, E.; **Veseljak, D.**; Mravinac, B.; Meštrović, N. Long-read genome assembly of the insect model organism *Tribolium castaneum* reveals spread of satellite DNA in gene-rich regions by recurrent burst events. *Genome Research* 2024 (in press) **[Q1] co-author**

Volarić, M.; Despot-Slade, E.; **Veseljak, D.**; Pavlek, M.; Vojvoda Zeljko, T.; Mravinac, B.; Meštrović, N. The Genome Organization of 5S rRNA Genes in the Model Organism *Tribolium castaneum* and Its Sibling Species *Tribolium freemani*. *Genes* 2024, 15, 776. <https://doi.org/10.3390/genes15060776> **[Q2] co-author**

Gržan, T.; Dombi, M.; Despot-Slade, E.; **Veseljak, D.**; Volarić, M.; Meštrović, N.; Plohl, M.; Mravinac, B. The Low-Copy-Number Satellite DNAs of the Model Beetle *Tribolium castaneum*. *Genes* 2023, 14, 999. <https://doi.org/10.3390/genes14050999> **[Q2] co-author**

Volarić, M.; Despot-Slade, E.; **Veseljak, D.**; Meštrović, N.; Mravinac, B. Reference-Guided De Novo Genome Assembly of the Flour Beetle *Tribolium freemani*. *Int. J. Mol. Sci.* **2022**, *23*, 5869. <https://doi.org/10.3390/ijms23115869> [Q1] co-author

Volarić, M.; **Veseljak, D.**; Mravinac, B.; Meštrović, N.; Despot-Slade, E. Isolation of High Molecular Weight DNA from the Model Beetle *Tribolium* for Nanopore Sequencing. *Genes* **2021**, *12*, 1114. <https://doi.org/10.3390/genes12081114> [Q2] co-author

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### Conference abstracts

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**Veseljak, D.**; Volarić, M.; Despot-Slade, E.; Meštrović, N.; Mravinac, B: The genomes of *Tribolium* sibling species framed by the evolution of satellite DNAs // Program and Abstracts, Arthropod Satellite Meeting, Helsinki 2024. Helsinki: EED organizing committees, 2024. str. 31-31 – **short talk**

**Veseljak, D.**; Despot-Slade, E.; Volarić, M.; Meštrović, N.; Mravinac, B: Satellitomes of flour beetles from the genus *Tribolium*: an evolutionary perspective // Euro EvoDevo 2004 Programme Book. Helsinki: EED organizing committees, 2024. str. 567-567 – **poster presentation**

Volarić, M.; Despot-Slade, E.; Meštrović, N.; Mravinac, B; **Veseljak, D.**: Oxford Nanopore Sequencing reveals complex mechanisms of repetitive DNA propagation in *Tribolium castaneum* // International congress on transposable elements 2024: Abstract book. Saint Malo: ICTE, 2024. str. 154-154 – **poster presentation**

**Veseljak, D.**; Mravinac, B: Exploring the satellitome of the North american flour beetle *Tribolium brevicornis* // 8th Faculty of Science PhD Student Symposium: Book of Abstracts. Zagreb: Faculty of Science, University of Zagreb, Zagreb, Croatia, 2024. str. 81-81 – **poster presentation**

**Veseljak, D.**; Despot-Slade, E.; Volarić, M.; Meštrović, N.; Mravinac, B: Dynamic evolution of satellite DNAs drastically alters genomes of *Tribolium* sibling species // Abstract Book: The Evolution of Animal Genomes. -: European Molecular Biology Organization (EMBO), 2023. str. 148-148 – **poster presentation**

**Veseljak, D.**; Mravinac, B: The satellitome of the confused flour beetle *Tribolium confusum*: genomic and cytogenetic aspects // 7th Faculty of Science PhD Student Symposium: Book of Abstracts / Pavlek, Katarina (ur.). Zagreb: Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu, 2023. str. 103-103 – **poster presentation**

**Veseljak, D.**; Mravinac, B: Satellitome analysis of the black flour beetle *Tribolium madens* // Book of Abstracts of the Congress of the Croatian Society of Biochemistry and Molecular Biology / Dulić, Morana; Sinčić, Nino; Vrhovac Madunić, Ivana (ur.). Zagreb: Hrvatsko društvo za biokemiju i molekularnu biologiju (HDBMB), 2022. str. 153-153 – **poster presentation**

**Veseljak, D.**; Mravinac, B: Satellitome characterization of the black flour beetle *Tribolium madens* // Simpozij studenata doktorskih studija PMF-a: knjiga sažetaka = 6th Faculty of Science PhD student symposium: Book of Abstracts / Schneider, Petra (ur.). Zagreb: Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu, 2022. str. 236-237 – **poster presentation**

Volarić, M.; **Veseljak, D.**; Mravinac, B; Meštrović, N.; Despot-Slade, E: Nanopore sekvenciranje kukaca roda *tribolium* s tvrdim egzoskeletom // 6. simpozij studenata doktorskih studija PMF-a: knjiga sažetaka = 6th Faculty of Science PhD student symposium: book of abstracts / Schneider, Petra (ur.). Zagreb: Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu, 2022. str. 238-239 – **poster presentation**

**Veseljak, D.**; Mravinac, B: Analysis of the satellitome of the flour beetle *Tribolium freemani* by high throughput sequencing // Simpozij studenata doktorskih studija PMF-a : knjiga sažetaka = PhD student symposium 2021 : book of abstracts / Barišić, Dajana (ur.). Zagreb: Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu, 2021. str. 263-264 – **poster presentation**

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### Short-term study visit

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23.10. - 05.11.2022. Two-week study visit to prof. Jiri Macas research group, Laboratory of molecular cytogenetics, Institute of plant molecular biology, České Budějovice, Czech Republic

## Workshops and webinars

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2024	“Inscape or vector graphics for everyone”, SRCE’s training programs, held online
2024	“Introduction to Galaxy platform”, SRCE’s training programs, held online
2022	“10th RepeatExplorer Workshop on the Application of Next Generation Sequencing to Repetitive DNA Analysis”, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic
2022	“Nanopore Community Meeting 2022”, Oxford Nanopore Technologies, held on-line
2022	“Academic Integrity – Plagiarism and how to avoid it“, Elsevier, on-line webinar
2022	“How to write and publish an article“, Elsevier, on-line webinar
2022	“How to find a journal wisely“, Elsevier, on-line webinar
2021	“9th RepeatExplorer Workshop on the Application of Next Generation Sequencing to Repetitive DNA Analysis”, held online
2021	“Eukaryotic Genome Assembly using PacBio and Hi C”, Physalia courses workshop, held online
2021	“An Insight Into Journal Editorial Processes“, Wageningen Academic Publishers, on-line webinar
2021	“Step-by-step Guide on How to Prepare a Successful Paper“, AD webinars Springer Nature, on-line webinar
2020	Winter School of Research Commercialization, Faculty of Pharmacy and Biochemistry and the Centre for Research, Development and Technology Transfer of the University of Zagreb, Zagreb, held on-line

## Grants and fellowships

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- EMBO travel grant for participation in "The Evolution of Animal Genomes" workshop (18.-21.2023., Sevilla, Spain)
- Institute Ruđer Bošković’s funding for short-term study visits of young researchers to laboratories abroad in 2022

## Science popularization

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2024	“How to become a scientist?“ workshop organisation for students of OŠ Ivana Rangera Kamenica
2022	Participation in Ruđer Bošković Institute Open Day
2015/16/17/18	Participation in the manifestation Biology Night, Univesity of Zagreb