

**Komarov Botanical Institute
Vavilov Institute of Plant Genetic Resources
RASA-Center, Peter the Great St. Petersburg Polytechnic University
St. Petersburg, Russia**

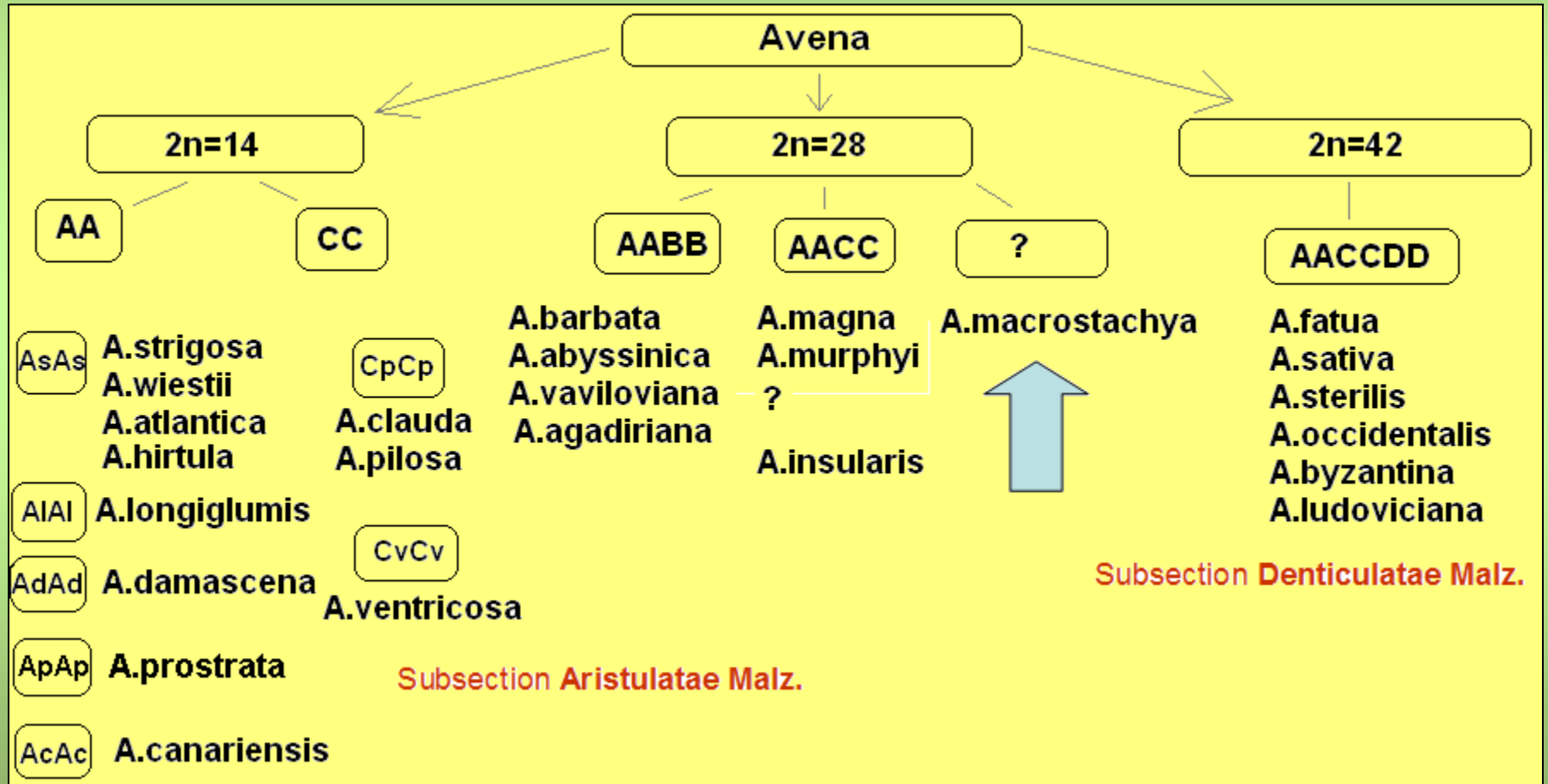
**Alexander V. Rodionov, Liubov M. Krainova, Elena S. Kim, Natalia B. Tyupa,
Julia V. Mikhailova, Alexander A. Gnutikov, Igor G. Loskutov**

**The origin of few polyploid *Avena*
species inferred from
an ITS polymorphism study**



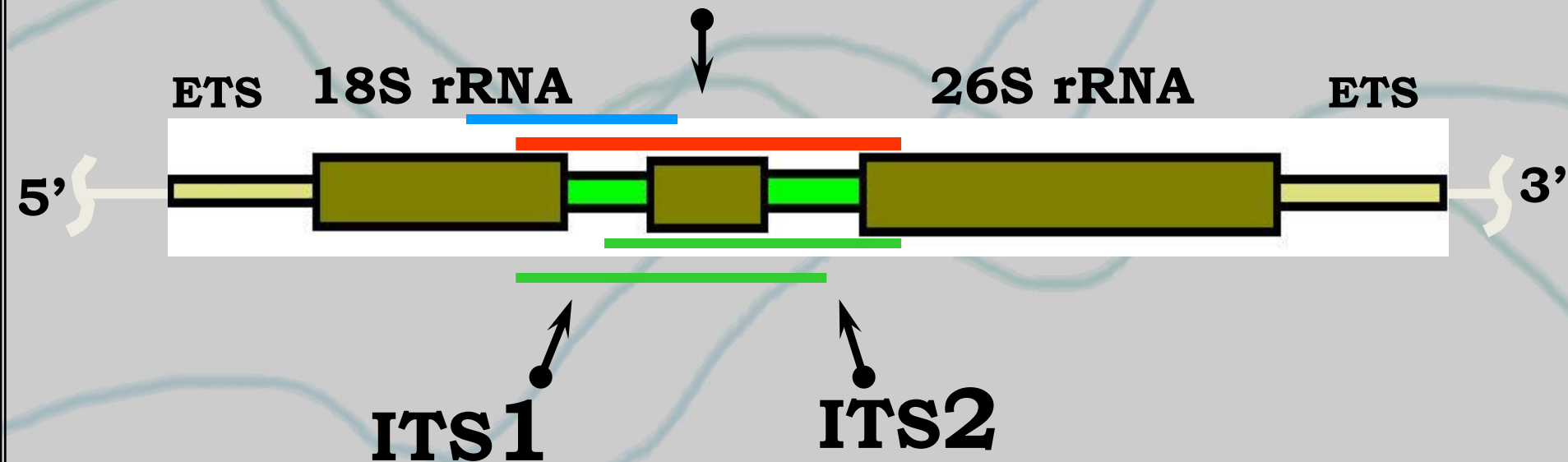
Traditional view on the genus *Avena*

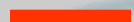
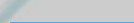
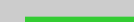
Section *Euavena* Griseb.



35S rRNA Gene

5.8S rRNA

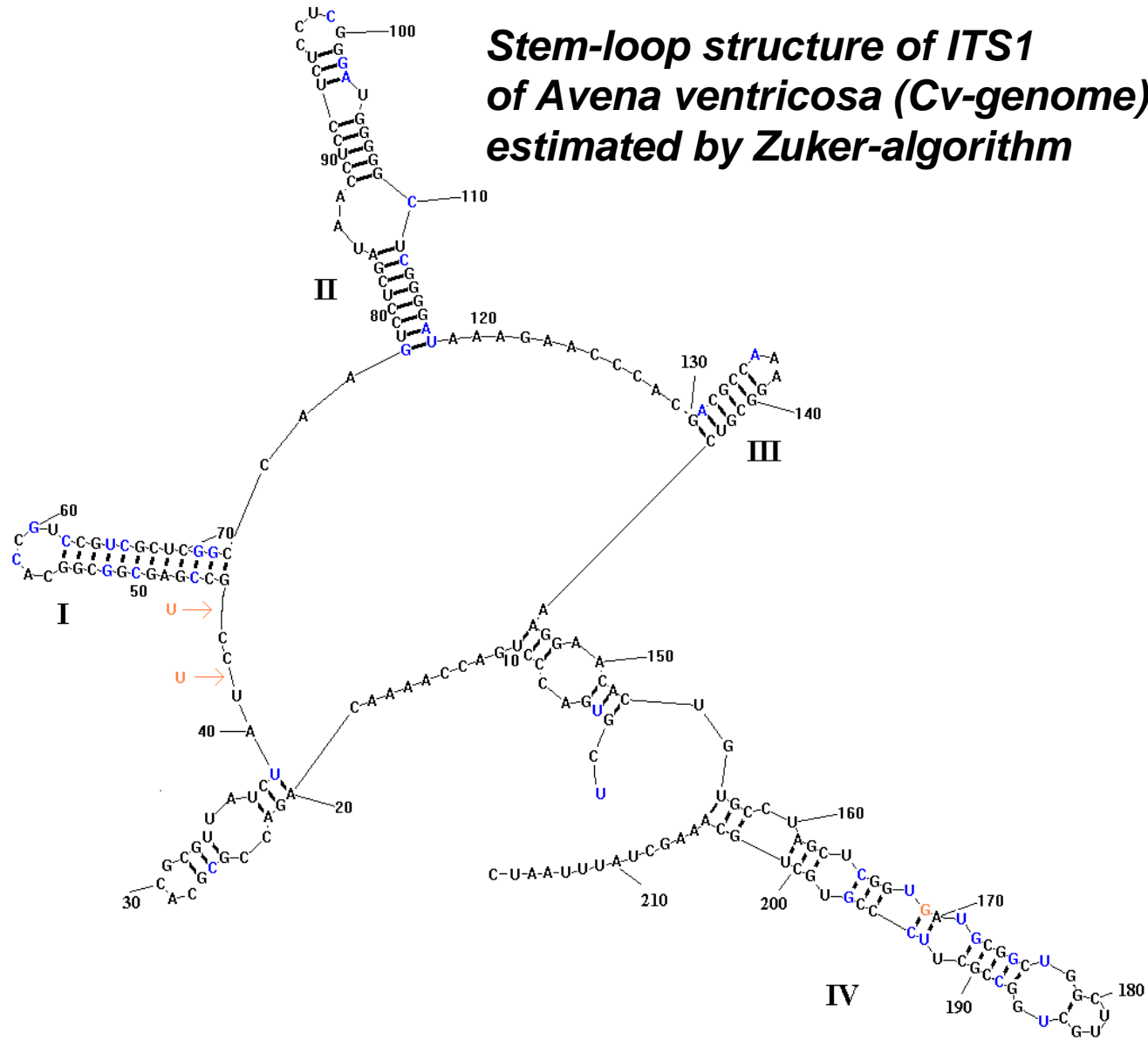


-  -fragment of rDNA that was sequenced by the Sanger sequencing method
-  fragment of rDNA that was sequenced by pyrosequencing system «454»
-  fragments of ITS1 and ITS2 that were sequenced by using C-genome specific primers (SCAR analysis)

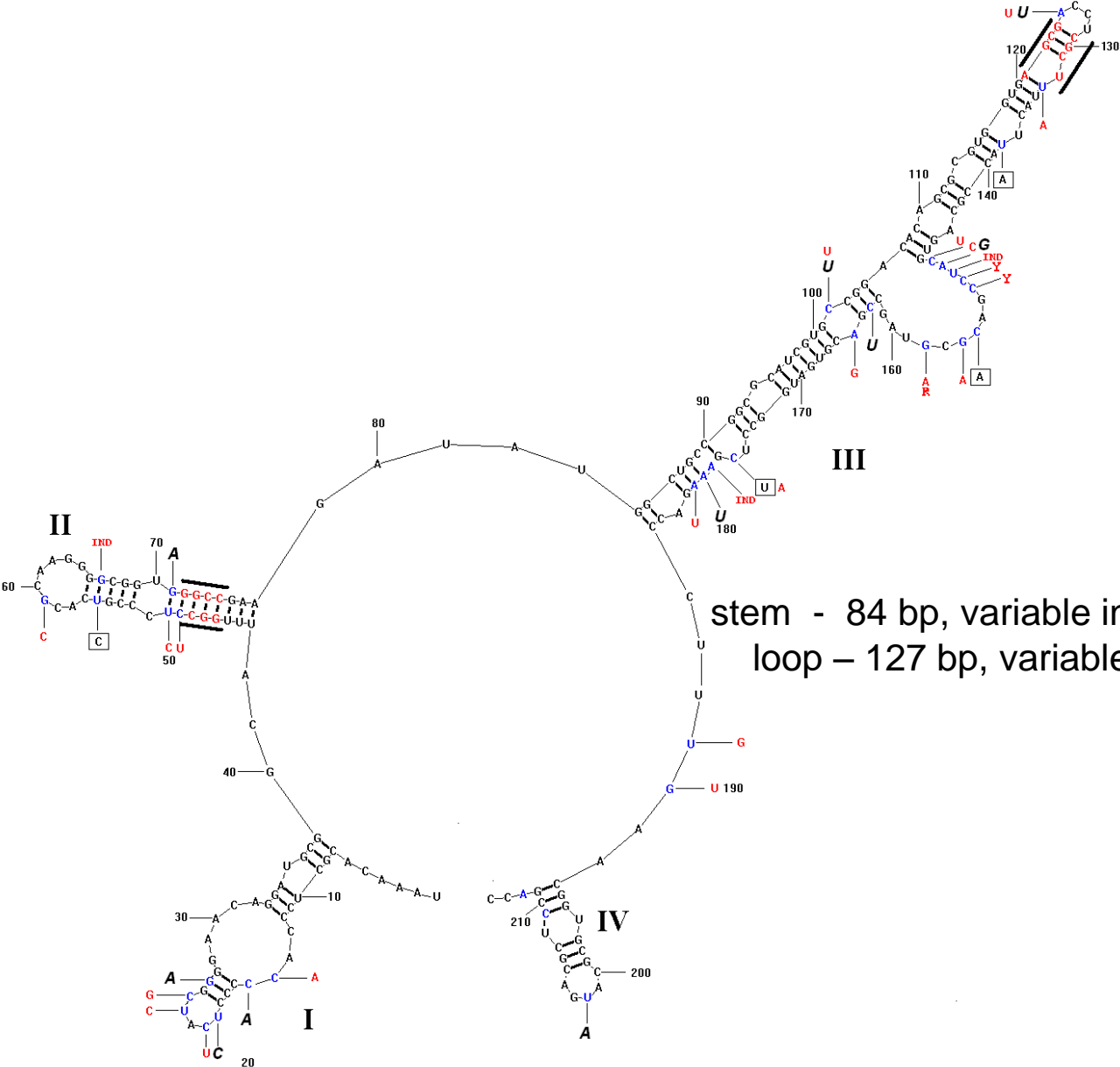
ITS – internal transcribed spacers 1 & 2

ETS – external transcribed spacer

Stem-loop structure of ITS1 of *Avena ventricosa* (Cv-genome), estimated by Zuker-algorithm



Secondary structure of the ITS2 transcript



stem - 84 bp, variable in *Avena* species – 23 positions
 loop – 127 bp, variable - 14 positions

Variable positions in ITS1 of the *Avena* species

Ancestral sequence for <i>Avena</i>		tctta-ttgcacgccgcccgggtctcagcctagggctcggcggttgctccgcca
	CmCm <i>A. macrostachya</i>c....a.....g.....t.t...c.t.ta....
}	Cv <i>A. ventricosa</i>-.....t.....ga..ataa...t.t.....t.....
	Cp <i>A. clauda</i>-Y.....t.....gaM...aa...t.t...c.t.t.K...
}	Cp <i>A. pilosa</i>-Y.....t.....gaM...aa...t.t...c.t.t.K...
	As <i>A. hirtula3</i>	.a...t...t.t..M.-..a.a...t.K.t.....t...aa.....t...
}	As <i>A. hirtula10</i>	.a...t...t.t..M.-..a.a...t.K.t.....t...aa.....t...
	As <i>A. atlantica</i>	.a.Y.t...t.t....-..aSa...t.t.t.....t...aa.....t...
}	As <i>A. strigosa</i>	.a.Y.t...t.t....-..a.a...t.t.t.....t...aa.....t...
	As <i>A. wiestii</i>	.a...t...t.t..Y.-t.a.a...t...t.....t...aa.....t...
}	Al <i>A. longiglumis</i>	Wa...t...t.t....-..a.a...t...t.....t...aa.....t...
	Ac <i>A. canariensis</i>	Wa...t...t.t....-..a.a...t...t.....t...aa...t...t...
}	Ap <i>A. prostrata</i>	.a...t...t.t...R-..a.a...t.t.t.....t...aRY.....t...
	Ad <i>A. damascena</i>	.a.Y.t...t.t....-..a.a...t.t.t.....t...aa.....t...
}	AB <i>A. barbata</i>	.a...t...t.t....-..a.a...t...t.....t...aa.....t...
	AB <i>A. abyssinica</i>	.a...t...t.t....-..a.a...t.K.t.....t...aa.....t...
}	AB <i>A. vaviloviana</i>	.a...t...t.t....-..a.a...t...t.....t...aa.....t...
	AC <i>A. magna</i>	.a...t...t.t....-..a.a...t...t.....t...aa.....t...
}	AC <i>A. murphyi</i>	.a...t...t.t....-..a.a...t...t.....t...aa.....t...
	?? <i>A. insularis</i>	.a...t...t.t....-..a.a...t.t.t.....t...aa.....t...
}	?? <i>A. agadiriana</i>	.a...t...t.t...a-..a.a...t.t.t.....t...aaY.....t...
	ACD <i>A. occidentalis</i>	.a...t..Rt.t....-..a.a...Y...t.....t...aa.....t...
}	ACD <i>A. fatua</i>	.a...t..at.t....-..a.a.....t.....t...aa.....t...
	ACD <i>A. ludoviciana</i>	.a...t...t.t....-..a.a...?..t.....t...aa.....t...
}	ACD <i>A. sterilis</i>	Wa...t..Rt.t.S.-..a.a...t...t.....t...aa.....t.M.
	ACD <i>A. sativa</i>	.a...t..Rt.t....-..a.a...Y...t.....t...aa.....t...
ACD <i>A. byzantina</i>	.a...t..Rt.t....-..a.a.....t.....t...aa.....t...	

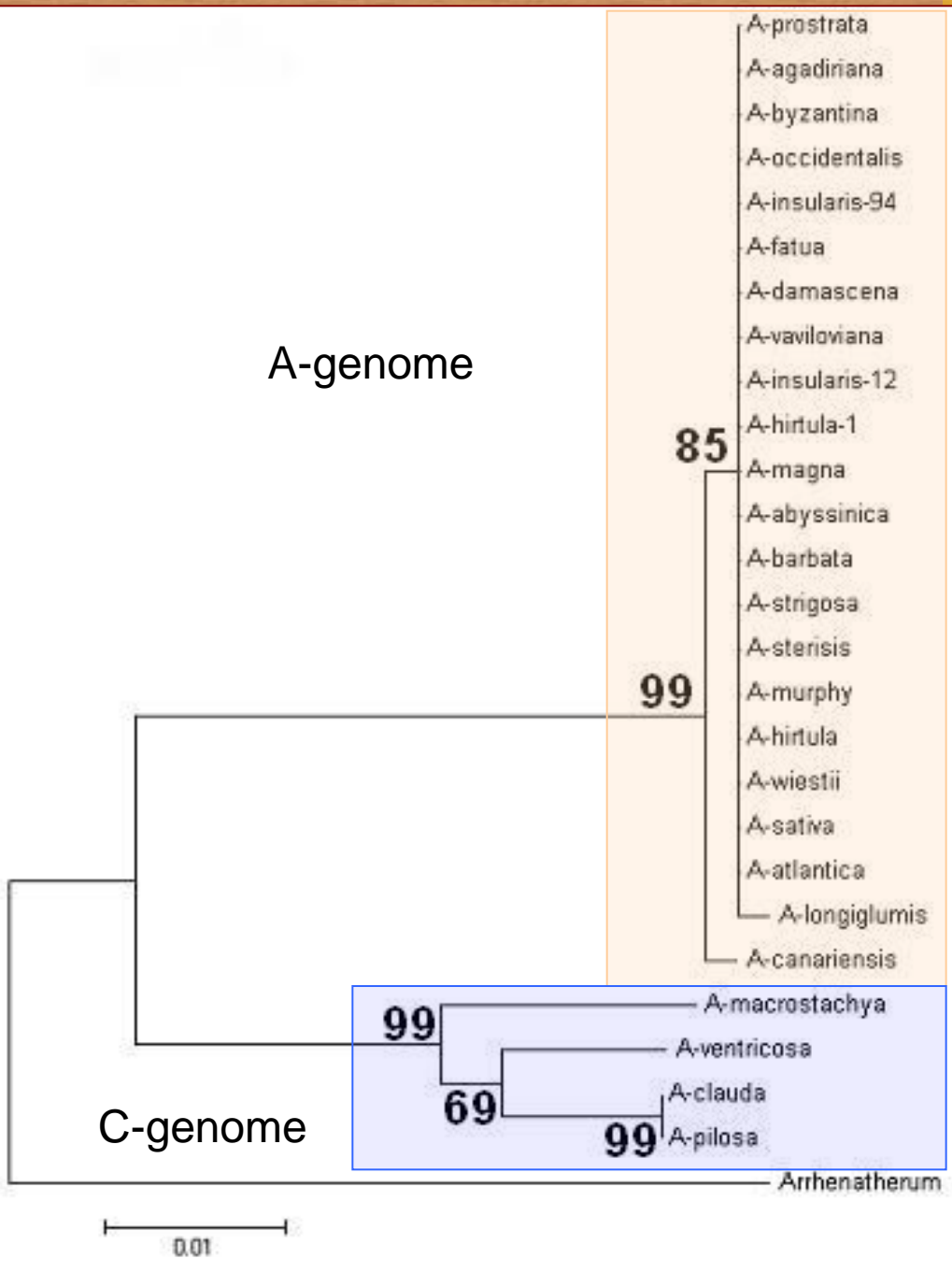
Sanger sequencing revealed only A-genome specific ribosomal DNA sequences in polyploid *Avena* species.

There is an elimination of the C-genome ribosomal DNA in genomes of polyploid oats.

**P-distance between Avena species (%)
(based on comparison of internal transcribed spacers)**

Вид	<i>macrostachya</i>	<i>clauda</i> (C)	<i>ventricosa</i> (C)	<i>pilosa</i> (C)	<i>longiglumis</i> (A)	<i>wiestii</i> (A)	<i>hirtula</i> (A)	<i>canariensis</i> (A)	<i>atlantica</i> (A)	<i>sterilis</i> (ACD)
<i>clauda</i> (C)	4,21									
<i>ventricosa</i> (C)	3,28	2,88								
<i>pilosa</i> (C)	3,72	0,89	2,19							
<i>longiglumis</i> (A)	9,19*	10,02	9,67	9,45						
<i>wiestii</i> (A)	9,19	9,35	9,01	8,79	1,31					
<i>hirtula</i> (A)	8,75	9,58	9,23	9,01	0,87	0,44				
<i>canariensis</i> (A)	8,77	9,38	9,25	8,81	0,87	1,09	0,87			
<i>atlantica</i> (A)	9,43	9,82	9,47	9,25	1,97	1,31	1,31	1,31		
<i>sterilis</i> (ACD)	9,19	10,02	9,67	9,45	0,65	1,31	0,87	0,87	1,97	
<i>sativa</i> (ACD)	8,75	9,35	9,23	8,79	0,87	1,09	0,87	1,09	1,75	0,65

* - Blue - p-distance between ITS1 and ITS2 of the C-genome and A-genome species



Phylogenetic tree of *Avena* species derived from analysis of ITS1 and ITS2 sequences.

***A. macrostachya* carries a C-genome ribotype**

56. <i>A. eriantha</i> _gi212004411 (2)	AGACCGCGCACGCGTT-ATCT-ATCC--GCTGGCGGG
57. <i>A. eriantha</i> _gi212004410 (2)	AGACCGCGCACGCGTT-ATCT-ATCC--GCTGGCGGG
58. <i>A. clauda</i> _gi212004394 (2)	AGACCGCGCACGCGTT-ATCT-ATCC--GCGGCGGG
59. <i>A. clauda</i> _gi212004393 (2)	AGACCGCGCACGCGTT-ATCT-ATCC--GCGGCGGG
60. <i>A. clauda</i> _gi212004392 (2)	AGACCGCGCACGCGTT-ATCT-ATCC--GCGGCGGG
61. <i>A. ventricosa</i> _gi212004508 (2)	AGACCGCGCACGCGTT-ATCT-ATCC--GCGGCGGG
62. <i>A. ventricosa</i> _gi212004507 (2)	AGACCGCGCACGCGTT-ATCT-ATCC--GCGGCGGG
63. <i>A. ventricosa</i> _gi212004506 (2)	AGACCGCGCACGCGTT-ATCT-ATCC--GCGGCGGG
64. <i>A. ventricosa</i> _gi212004505 (2)	AGACCGCGCACGCGTT-ATCT-ATCC--GCGGCGGG
65. <i>A. ventricosa</i> _VIR_k-1909_gi41394443 (2)	AGACCGCGCACGCGTT-ATCT-ATCC--GCGGCGGG
66. <i>A. wiestii</i> _gi28932773 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
67. <i>A. prostrata</i> _7197_gi119514749 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
68. <i>A. damascena</i> _gi212004402 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
69. <i>A. damascena</i> _gi212004401 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
70. <i>A. longiglumis</i> _VIR_k-1811_gi41394442 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
71. <i>A. longiglumis</i> _9071_gi119514697 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
72. <i>A. canariensis</i> _gi212004386 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
73. <i>A. canariensis</i> _gi212004385 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
74. <i>A. hirtula</i> _gi212004427 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
75. <i>A. hirtula</i> _gi212004426 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
76. <i>A. strigosa</i> _VIR_K-9888_gi89275077 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
77. <i>A. strigosa</i> _19531-1_gi119514705 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
78. <i>A. atlantica</i> _VIR-1894_gi85062658 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG
79. <i>A. atlantica</i> _gi212004363 (2)	AGACCGAGCAGCGCGTT-ATCT-ATTCGCTGGGTGG

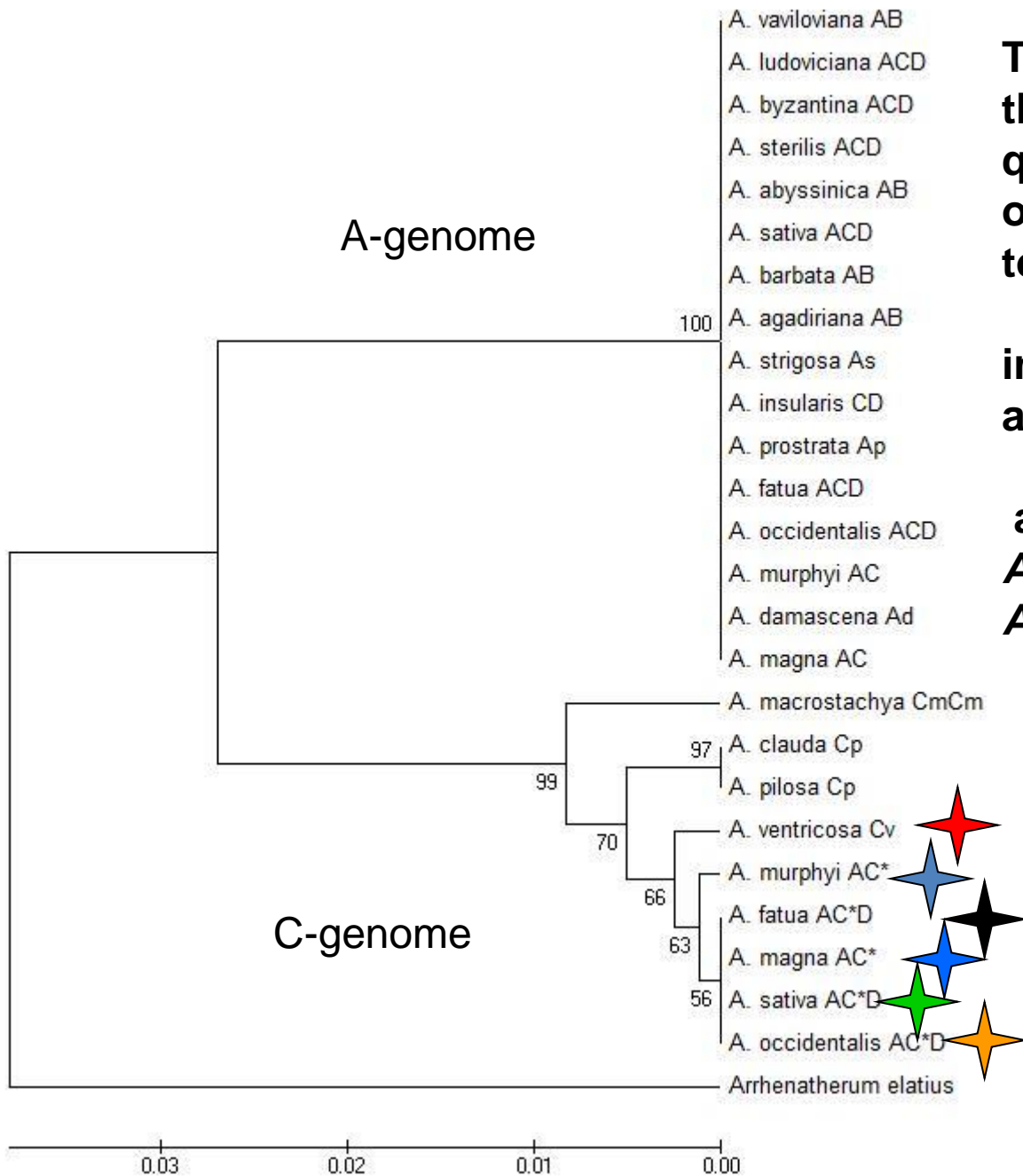
To reveal the origin of the C-genome in polyploid oats we, basing on the ITS1 and ITS2 sequences of *Avena* species, (Rodionov et al., 2005), designed C-genome ITS specific primers:

M13R-cAvena ITS1F

and

M13R-cAvena ITS2R.

14. <i>A. macrostachya</i> _gi223006478	ATGGCCCTGATAGACCCTT--TGAACGG--TGCGCATTAGCC
15. <i>A. eriantha</i> _VIR_k-201_gi42563598	ATGGCCCTCG-TAGACCCTT--TGAACGG--TGCGCATTAGCC
16. <i>A. eriantha</i> _gi212004411	ATGGCCCTCG-TAGACCCTT--TGAACGG--TGCGCATTAGCC
17. <i>A. clauda</i> _gi212004394	ATGGCCCTCG-TAGACCCTT--TGAACGG--TGCGCATTAGCC
18. <i>A. clauda</i> _gi212004393	ATGGCCCTCG-TAGACCCTT--TGAACGG--TGCGCATTAGCC
19. <i>A. ventricosa</i> _gi212004508	ATGGCCCTCG-TAGACCCTT--TGAACGG--TGCGCATTAGCC
20. <i>A. ventricosa</i> _gi212004506	ATGGCCCTCG-TAGACCCTT--TGAACGG--TGCGCATTAGCC
21. <i>A. prostrata</i> _7191_gi119514747	ATGGCCCTAGAAAAGACCCTT--GTAAACGGTIS-GCGCATTAGCC
22. <i>A. prostrata</i> _7060_gi119514688	ATGGCCCTAGAAAAGACCCTT--GTAAACGGTTC-GCGCATTAGCC
23. <i>A. damascena</i> _gi212004402	ATGGCCCTAGAAAAGACCCTT--GTAAACGGT--GCGCATTAGCC
24. <i>A. damascena</i> _gi212004401	ATGGCCCTAGAAAAGACCCTT--GTAAACGGT--GCGCATTAGCC
25. <i>A. longiglumis</i> _VIR_k-1811_gi41394442	ATGGCCCTAGAAATGACCCTT--GTAAACGGT--GCGCATTAGCC
26. <i>A. longiglumis</i> _9071_gi119514697	ATGGCCCTAGAAATGACCCTT--GTAAACGGT--GCGCATTAGCC
27. <i>A. canariensis</i> _gi212004386	ATGGCCCTAGAAAAGACCCTT--GTAAACGGT--GCGCATTAGCC
28. <i>A. canariensis</i> _gi212004385	ATGGCCCTAGAAAAGACCCTT--GTAAACGGT--GCGCATTAGCC
29. <i>A. wiestii</i> _gi28932773	ATGGCCCTAGAAATGACCCTT--GTAAACGGT--GCGCATTAGCC
30. <i>A. hirtula</i> _gi212004427	ATGGCCCTAGAAATGACCCTT--GTAAACGGT--GCGCATTAGCC
31. <i>A. hirtula</i> _gi212004426	ATGGCCCTAGAAATGACCCTT--GTAAACGGT--GCGCATTAGCC
32. <i>A. atlantica</i> _VIR-1894_gi85062658	ATGGCCCTAGAAAAGACCCTT--GTAAACGGT--GCGCATTAGCC
33. <i>A. atlantica</i> _gi212004363	ATGGCCCTAGAAATGACCCTT--GTAAACGGT--GCGCATTAGCC



This approach has yielded the presence of minor quantities of C-genomic rDNA of an ancestor specie, related to *Avena ventricosa* (★),

in the tetraploids *A. magna* and *A. murphyi*

as well as in the hexaploids *A. sativa*, *A. occidentalis* and *A. fatua*.

Our results were correspondent with the results of N.Nikoloudakis and A.Katsiotis, who used another pair of C-genome specific primers

There are only 1-3% of C-genome specific ribosomal DNA loci in allopolyploid *Avena* genomes

	General number of sequenced ITS1	C-genome specific	A-genome specific
<i>Avena insularis</i> (2067, Sicily);	827 100%	24 2.9%	803 97.1
<i>Avena ludoviciana</i> (389, Venesuella); 1	790 100%	14 1.8%	776 98.2%
<i>Avena ludoviciana</i> (389, Venesuella); 2	757 100%	13 1.7%	744 98.3%
<i>Avena fatua</i> (30, Tuva);1	873 100%	21 2.4%	852 97.6%
<i>Avena fatua</i> (30, Tuva);2	777 100%	19 2.4%	759 97.6%
<i>Avena sterilis</i> (511, Israel);1	766 100%	10 1.3%	756 98.7%
<i>Avena sterilis</i> (511, Israel);2	827 100%	13 1.6%	814 98.4%



Alexander Rodionov



Lena Kim and Yulia Mikhailova

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Natasha Tyupa



Alexander Gnutikov



Igor Loskutov



THANKS !