CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES
OF WILD FAUNA AND FLORA

Thirtieth meeting of the Animals Committee
Geneva (Switzerland), 16-21 July 2018

BLACK SEA BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS PONTICUS*) [DECISION 17.300]

The attached information document has been submitted by Ukraine in relation to agenda item 23*.

* *The geographical designations employed in this document do not imply the expression of any opinion whatsoever on the part of the CITES Secretariat (or the United Nations Environment Programme) concerning the legal status of any country, territory, or area, or concerning the delimitation of its frontiers or boundaries. The responsibility for the contents of the document rests exclusively with its author.*

Expert opinion

according to the results of the analysis of the microsatellite loci of the genome of two dolphins (*Tursiops truncatus*) that belongs to Privet Company "Afalina T".

At the Department of Genetics and Molecular Biology of the Odessa National I. I. Mechnikov University carried out molecular-genetics analysis of the alleles of microsatellite loci of the genomes two dolphins (*Tursiops truncatus*) from Black Sea.

The DNA was isolated from blood samples of two dolphins with the personal names "George" (No5) and "Zevs" (No6). The blood samples for genetic analysis have been provided by Privet Company "Afalina T", which has registered at address: st. Porika, 7, p. Shiroka Grieble, Vinnitsa region, Ukraine. Isolation of DNA was performed according to the method - «Isolation of DNA using Chelex 100» (Sivolap and Kryvda, 2001). As a control it was taken DNA of dolphin, which have been died on the Black Sea coast (beach "Longeron" (Odessa, Ukraine)).

The primers (Table 1) to *Tursiops*-specific loci for analysis of genetic polymorphism were synthesized by Metabion (Germany) and applied as recommended (Krützen et al. 2001; Sumiyama et al., 2008; Richards et al., 2013).

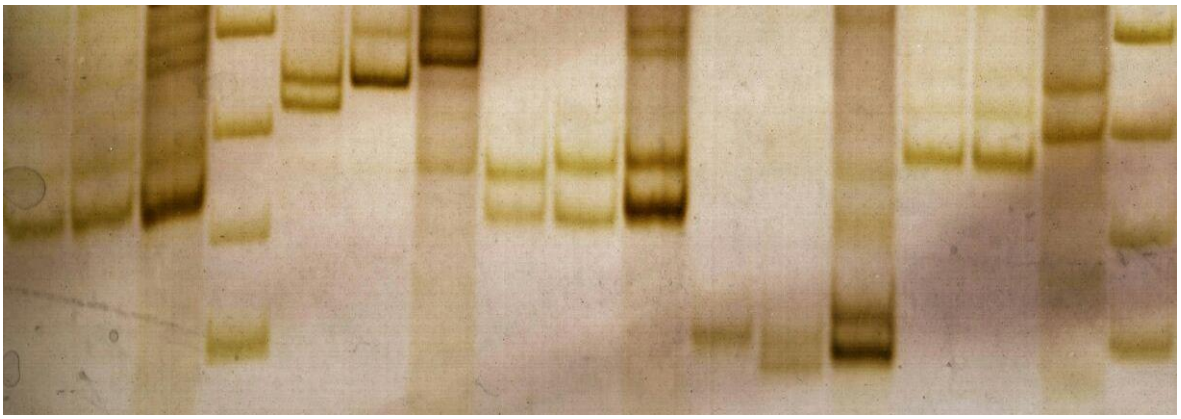
PCR analysis was performed according to Krützen et al. (2001) and Sumiyama et al. (2008). PCR has been done on Flex Cycler thermocycler (Analytik Jena, Germany). Amplification products have been analyzed by electrophoresis in 7% polyacrylamide gel (PAAG) (Fig. 1, Table 2) by using VE-20 (Helicon, Russia). Electrophoresis passed at a voltage of 300 B, for 2.5 hours. For visualization of amplification fragments in gels, polyacrylamide gels were stained with $AgNO_3$ in accordance with the recommendations (Promega, 1999). The size of amplification fragments (alleles of microsatellite loci) have been determined by comparing with molecular weight marker pUC 19/Msp I by using computer program GelAnalyzer.

Table 1

Sequences of primers to microsatellite loci in genome *Tursiops truncatus* according to Krützen et al. (2001), Sumiyama et al. (2008), Richards et al., (2013)

Locus	Repeat sequence	Sequence of primers 5' – 3'	Alleles size (bp.)
MK3	(A)9TAC(GT)15AT(GT)7	tgcattcatgtaaaggtgcg	139-171
		ctgcaactagagaaagcccg	
MK5	TG)13CT(TG)2CA(TG)2 (TA)2(TG)4	ctcagagggaaatgaggctg	201-221
		tgtctagaggtcaaagccttcc	
MK6	(GT)17	gtcctctttccaggtgtagcc	145-189
		gcccactaagtatgttgcagc	
MK8	(CA)23	tcctggagcatcttatagtggc	87-119
		ctctttgacatgccctcacc	
MK9	(CA)17	cataacaaagtgggatgactcc	168-180
		ttatcctgttggctgcagtg	

D-18	(CA)3-TA-(CA)21	cccaaaaccgacagacagac	90
		gatctggggatgcagg	
Ev-37	(AC)24	agcttgatttggaagtcatga	178-224
		tagtagtgccgtgataaagtgc	



1 2 3 M 4 5 6 7 8 9 10 11 12 13 14 15 M

Fig. 1. Electrophoresis of PCR fragments of microsatellite loci in PAAG: (***MK3***) **1** - № 5, **2** - № 6, **3** - Control; **(*MK5*) 4** - № 5, **5** - № 6, **6** - Control; (***MK6***) **7** - № 5, **8** - № 6, **9** - Control; (***MK8***) **10** - № 5, **11** - № 6, **12** - Control; (**MK9**) **13** - № 5, **14** - № 6, **15** -Control, **M** - pUC 19 / Msp I.

Table 2

Alleles of microsatellite loci of investigated dolphins that have been revealed by PCR

№	Locus	Alleles of microsatellite loci (bp)		
		"George" (No5)	"Zevs" (No6)	Control DNA of dolphins
1	***MK3***	153 - 153	153 - 153	153 - 153
2	***MK5***	204 - 217	217 - 217	-
3	***MK6***	153 - 171	153 - 173	155 - 175
4	***MK8***	113 - 113	107 - 113	109 - 119
5	***MK9***	178 - 178	178 - 178	190 - 216
6	***D-18***	94 - 94	94 - 94	97 -97
7	***Ev-37***	-	209 - 225	186 - 195

According to the data of microsatellite analysis of DNA dolphins "George" (No5) and "Zevs" (No6) we have revealed:

at microsatellite locus ***MK3*** dolphins "George" (No5) and "Zevs" (No6) are homozygous and characterized by alleles with the size of 153 bp both;

at locus ***MK5*** dolphin "George" (No5) is a heterozygous and characterized by alleles size 204 bp and 217 bp, dolphin "Zevs" (No6) is homozygous and characterized with allele - 217 bp;

at locus ***MK6*** dolphin "George" (No5) is a heterozygous, characterized by alleles 153 bp and 171 bp, dolphin "Zevs" (No6) is also a heterozygote and has alleles 153 bp and 173 bp;

at locus ***MK8*** dolphin "George" (No5) is homozygous, has allele 113 bp; dolphin "Zevs" (No6) is heterozygous and characterized by alleles 107 bp and 113 bp;

at locus ***MK9*** dolphins "George" (No5) and "Zevs" (No6) are homozygous and characterized by alleles 178 bp;

at locus ***D-18*** dolphins "George" (No5) and "Zevs" (No6) are homozygous and characterized by alleles size 94 bp;

at ***Ev-37*** locus of dolphin "Zevs" (No6) is heterozygous and characterized by alleles size 209 - 225 bp.

Thus, according to the data of microsatellite analysis it can be assumed that dolphins "George" (No5) and "Zevs" (No6) are sibs, that are, the descendants of one parent, due to the fact that they have the same alleles in a number of studied loci and do not have any cases when two dolphins have not at least one common allele among the investigated microsatellite loci. Also DNA of these two dolphins differed from the control sample DNA for all microsatellite loci except ***MK3.*** On the base of obtained data molecular-genetic analysis - alleles of microsatellite loci of genomes dolphins "George" (No5) and "Zevs" (No6), can be used as genetic passport data for these individuals, since the profiles of genotypes of these dolphins are unique for each individual and allows to distinguish these individuals from other dolphins of the Black Sea.

Head of the Department of Genetics and Molecular Biology
Odessa National I.I. Mechnikov University,
Prof., D.Sc. S.V. Chebotar

From: Домашлінець Володимир Григорович <domashlinets@menr.gov.ua>
Sent: Friday, 1 June, 2018 1:47 PM
To: Daniel Kachelriess <daniel.kachelriess@un.org>
Subject: Implementation of Decision 17.299, information for the AC30

Dear Mr Kachelriess,

In addition to the previous correspondence please find below a summarized information concerning implementation of Decision 17.299 on the Black Sea bottlenose dolphins (*Tursiops truncatus ponticus*)

1. Regarding para. a) of the Decision 17.299.

The CITES Management Authority (MA) has received an application for export of two individuals of *Tursiops truncatus ponticus* to UAE. Following CITES provisions and as per request of the CITES MA of Ukraine the relevant CITES Scientific Authority provided with non-detrimental finding and requested the Department of Genetics and Molecular Biology of Odessa National I.I. Mechnikov University to conduct the genetic analysis of the specimens above in accordance with Decision 17.299. The Department provided his advise which was sent to the CITES Secretariat and may be shared with the Animal Committee (please find it attached).

As there is no a kind of international protocol for such genetic studies approved in the framework of CITES the Odessa University did the genetic analysis based on their experience and knowledge. It worth to note that further elaboration of internationally agreed protocol or guidelines on genetic analysis of the dolphins would facilitate proper implementation of the Decision 17.299.

2. Regarding para. b) of the Decision 17.299.

Currently relevant genetic data of *Tursiops truncatus ponticus* are stored nationally because until now there is no a kind of intergovernmental agreement which can specify who, how and where will store the genetic profiles of the dolphins regionally.

3. Regarding para. c) of the Decision 17.299.

   A decision on issuance of export permit for the dolphins mentioned in p. 1 above is not taken yet. Since CITES COP17 no other export permits for *Tursiops truncatus ponticus* are issued.

4. With regards to comments of the CITES Secretariat in para. 9 of the document AC30 Doc. 23.

   Upon checking of CITES documentation for 2014 it should be noted that three wild specimens of *Tursiops truncatus* which were intended for export from Ukraine to Thailand are subspecies *T. t. gilli* rather than *T. t. ponticus* and originated from Japan.

   Sincerely yours,

Dr Volodymyr Domashlinets
Head of Wildlife Protection Unit
Department of Biodiversity Protection and Biosafety
Ministry of Ecology and Natural Resources of Ukraine
CITES Management Authority