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Case Report

Identification of the skeletal remains of the Czech communist regime crime victim, priest Josef Toufar

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ABSTRACT

Parish priest Josef Toufar died as a direct consequence of torture committed by Communist State Security Service agents, forcing him to confess that “miraculous” movement of crucifix above the main altar during the Holy Mass held in the Roman-Catholic church in Číhošť was staged by using a technical equipment. Josef Toufar was presumably buried in a mass grave at the cemetery in Prague-Ďáblice under a false name Josef Zouhar. In 2013 the Czech Bishops' Conference grant an approval to begin the process of his beatification. However, the beatification required the exhumation and identification of the remains.

In this case report, we describe the process of searching, exhumation, and the combined A-STR/Y-STR DNA analysis of remains of Pater Josef Toufar. His identification was feasible due to kinship analysis: buccal swabs of three family members (niece, grand-niece, and grand-nephew) were available for testing.

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1. Introduction

In the former Czechoslovakia, 83,000 persons were sentenced and 227 persons were executed by communist regime between 25.2.1948 and 29.12.1989 in fabricated political trials <http://www.policie.cz/clanek/obeti-komunistickeho-rezimu.aspx?q=Y2hudW09Mg%3d%3d>. However, other hundreds of persons were killed while trying to escape through iron curtain <https://www.ustrcr.cz/uvod/dokumentace-usmrcenych-statni-hranice/> or as a consequence of interrogation (i.e. bishop Josef Hlouch, cardinal Štěpán Trochta, philosopher Jan Patočka, and attorney Pavel Wonka). One of the victims of communist regime without identification and adequate burial was also Pater Josef Jindřich Toufar (Fig. 1).

According to the testimony of 19 parishioners in the local Church of the Assumption of the Virgin Mary in Číhošť, on 11th of December, 1949, during the Holy Mass the third Sunday of Advent, half a meter tall crucifix above the tabernacle on the main altar moved. Parish priest Josef Toufar reported this incident to the authorities. Communist State Security Service exploited the

situation as an excuse to religious repression and propaganda. State police (StB) officers 28.1.1950 kidnapped P. Josef Toufar from Číhošť, imprisoned him in Valdice jail and forced him by battering and thirst to sign the confession that miracle was staged using cables and leverage. This accusation was not substantiated by any evidence and crucifix movement was never reasonably explained.

For the filming propaganda film “Woe to him by whom the offense cometh!”, in a sorry state after repeated torture during interrogation, with ruptured gastric ulcer, Toufar was taken back to his church for camera shot on the pulpit. After surgery of peritonitis Toufar died the following day (25.2.1950) while his death was kept secret for four years even from his relatives. Toufar niece Marie Pospisilova was searching for his remains since 1954. Recently, evidence accumulated that he may have been buried in a mass grave at the Ďáblice cemetery, shaft no. XVI, under a false name Josef Zouhar. In April 2013, the Czech Bishops' Conference gave approval to begin the process of beatification of Josef Toufar, which required the exhumation and identification of the remains.

Between 1943 and 1961, 70 mass graves with 4 layers of 10 coffins each were used in Ďáblice cemetery for burial of unidentified dead, aborted fetuses, still born children, and anatomic and pathological waste [1]. In the course of Nazi occupation, active participants of the anti-fascist resistance have been buried here. After WWII, the cemetery was used for burial of convicted and executed Germans and their quislings. After

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¹ This paper is dedicated to the memory of our wonderful colleague, Dr. Markéta Šaňková, who participated in this project from its start and recently passed away.

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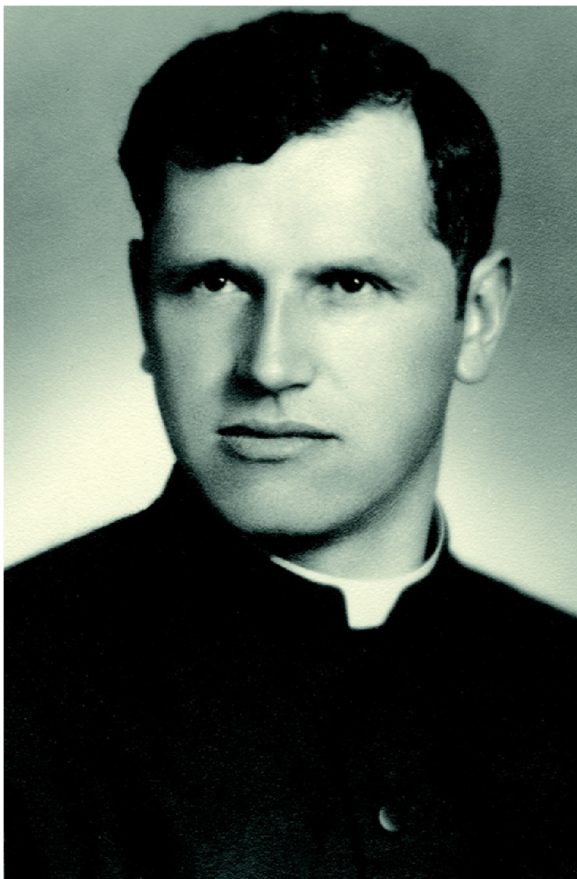


Fig. 1. Josef Toufar.

communist takeover in February 1948, the cemetery was used for the victims of fabricated political processes. Contrary to other shafts, a copy of burial record of shaft no. XVI was preserved.

In this case report, we describe the process of searching, exhumation, and identification of remains of Pater Josef Toufar through the DNA profiling and analysis of kinship in a broader family tree.

2. Materials and methods

2.1. Consent

The identification of the skeletal remains was conducted in compliance with Czech laws, with the approval of Pater Toufar's family and under the guidance of the Bishop of Diocese Hradec Králové, Mayor of the Capital City of Prague, National Heritage Institute, Ministry of Regional Development, and Prague Cemeteries Directorate. The family pedigree was drawn up with the assistance of Mr. Toufar's family members (Fig. 2) and all available living persons from pedigree were contacted, following recommendation no. 4 of DVI guidelines [2]. An informed consent for buccal swab analysis was obtained from living relatives of Josef Toufar: niece BN, grand-niece JC, and grand-nephew LT.

Persons with initials were available for mouth swabbing and DNA analysis. JT? is a proband, presumed to stand for Josef Toufar.

2.2. Archeological research

Identification of the skeletal remains was an integral part of the archeological research of mass graves, carried out in accordance

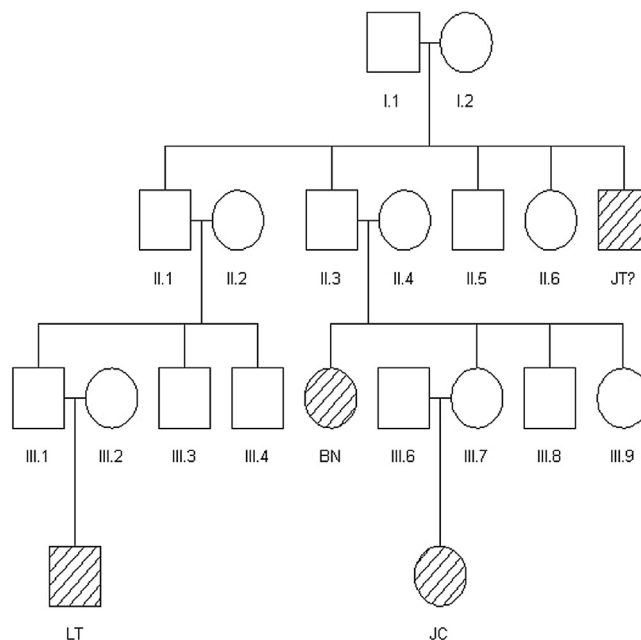


Fig. 2. Toufar pedigree.

with Act no. 256/2001 Coll., on Regulations and Rules on Funerals and Cemeteries and on Amendments to Certain Other Acts, as Amended. Owing to the fact that the Ďáblice cemetery is not only a currently used burial ground, but also a National cultural heritage, archeological works was approved in compliance with Act no. 20/1987 Coll., on State Landmark Conservation, as Amended not only by the operator of the burial ground and Archaeological Institute, but also by the National Heritage Institute.

In November 2014, three potential burial pits were probed. The third one was successful. The coffin (Fig. 3) was discovered with the help of the uniquely preserved funeral register for mass grave no. XVI from year 1950 under two later burials at a depth of 1.7 m below the current ground level. The remains of body were recovered by the team of anthropologists from the National Museum, archeologists from the National Heritage Institute, coroner, and forensic genetics expert (details to be published elsewhere).

Standard procedure for the archeological excavation was applied with the use of geodetic methods, photogrammetry, photography, and contextual information recording (Fig. 4).



Fig. 3. Mass grave in Ďáblice cemetery.

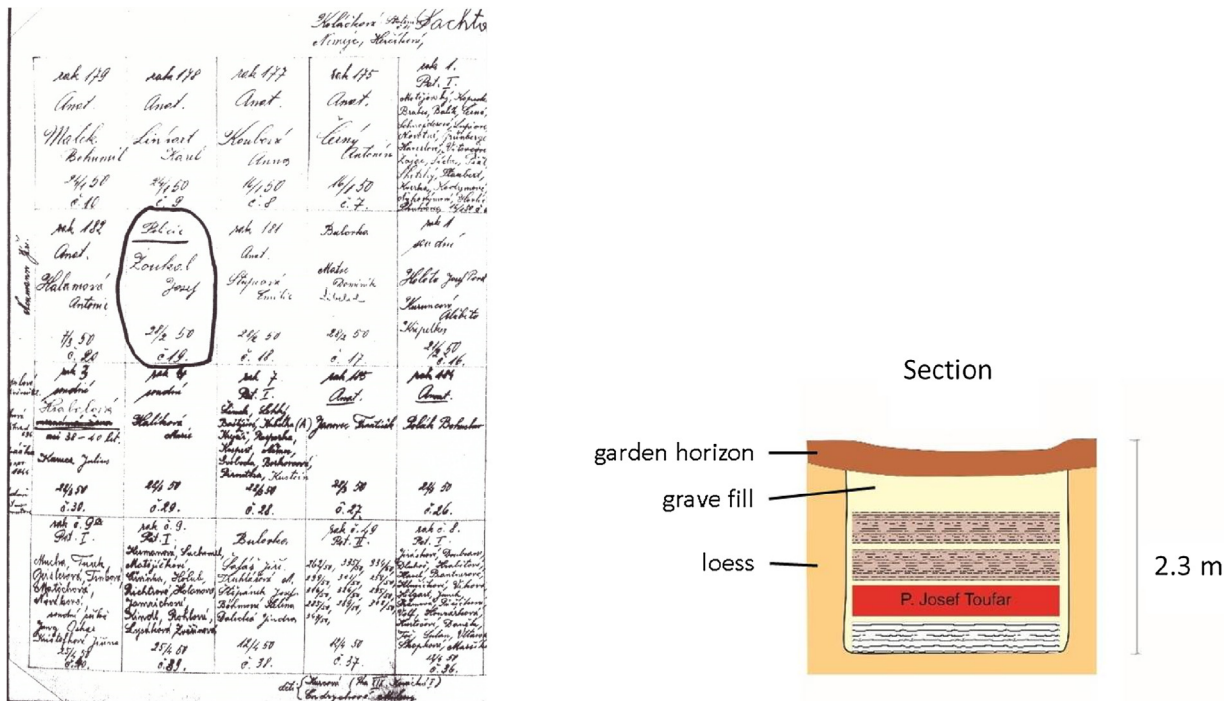


Fig. 4. Burial record of shaft no. XVI. On the left side (a), part of a document from funeral register for mass grave no. XVI with the record of J. Toufar's interment on 28.2.1950. Josef Zoukal name is circled (in other documents referred as Zouhar). On the right side (b), mass grave no. XVI – schematic section showing the position of J. Toufar's coffin.

The skull was completely fragmented and therefore it was not possible to make use of super-projection of the skull into photography as part of identification. The morphology of the skeleton corresponds uniquely to the male sex, age between 40 and 50 years. The height of his stature was between 170–175 cm [3]. Moreover, based on the evident anatomical skull dissection and presence of rubber drain nearby *os sacrum*, both confirmed in literature for Toufar body *post-mortem*, we set the non-genetical prior probability of Pater Toufar remains to be at least 50%.

2.3. DNA extraction from buccal swabs

DNA from buccal swabs BN, JC, LT, and persons handling biological materials was extracted using NucleoSpin Tissue kit from Macherey-Nagel according to manufacturer's instructions. Extraction was done in series to decrease the risk of contamination.

2.4. DNA extraction from left thigh bone

In the dedicated room, curved block 5.5 cm × 2.0 cm was excised by laboratory with a previous experience with decomposed bone samples from the left thigh bone with a bone saw [2].

Fragment was mechanically cleaned with DNA-free milling cutter, washed in 96%, exposed to UV light for 15 min each side, and dried in a closed laminar flow cabinet overnight. Its half was powdered using a bonemill (Laarmann Group BV, Roermond, The Netherlands) [4]. The second half was archived (Fig. 5).

DNA from 4 g of bone powder was extracted in three replicates using NucleoSpin Trace kit from Macherey-Nagel. On the left side, the whole thigh; on the right side, cleaned fragment before DNA extraction.

2.5. DNA quantification

The quantity of nuclear DNA from the bone extracts was determined using a real-time PCR approach Plexor HY (Promega, USA) according to Ref. [5] on a 7500 Fast Real-Time PCR System (Life Technologies (LT), Carlsbad, CA, USA).

2.6. DNA profiling

DNAs from proband bone, niece BN, grand-niece JC, and grand-nephew LT were profiled using PowerPlex ESX17 (Promega, USA) as described in manufacturer's instructions. We expected that if



Fig. 5. Purported left thigh bone of Josef Toufar.

full profile of bone sample would be obtained then the evidence for kinship would be sufficient. Our expectations were based on results of simulation. Simulating the true Toufar relationship in Familias software with loci of PowerPlex ESX17, 32.9% of the 1000 simulations with seed 12,345 were above the limit of likelihood ratio (LR)=400 that would increase 50% prior to more than 99.75% what is, in accordance with Hummel table [6], accepted by the Czech courts as kinship practically proven. Median of simulated LR was 83 and mean of simulated LR was 7710.

To deal with 66% chance that LR would not reach 400, samples of presumed Josef Toufar and his grand-nephew LT were genotyped for Y-STR using PowerPlex Y23 (Promega) as well.

2.7. Interpretation

Familias 3.2.1 beta [7] and the Czech population frequencies [8] were used for calculation of A-STR likelihood ratio for kinship and for simulations, following ISFG recommendations for paternity testing [9]. Y-STR likelihood ratio was calculated online <https://yhrd.org/kinship> using published approach [10]. Though our subjective prior was really high, to be conservative, prior probability of kinship was by default set to 50%. Other possible priors were dealt with in tabular form (Table 2). Other tunable parameters were set to: theta = 0; mutation probability = 0; silent alleles probability = 0; generation parameter = 1; maximum generations = 5; inbreeding parameter = 1; promiscuity parameter = 1; probability of dropout = 0 (for explanation see <http://familias.name/manual.pdf>) (Fig. 6).

3. Results

The full A-STR DNA profile was obtained for all control DNAs from buccal swabs while *D18S51* locus dropped out in bone sample. Also in bone sample, allelic dropout was possible at loci *TH01*, *D2S441*, *FGA*, *D8S1179* because only one allele per locus was found there. However, dropout at four loci would not have impacted the calculation of LR because bone sample alleles *TH01* 6, *D2S441* 11, *FGA* 24, *D8S1179* 12 were present in presumed Toufar relatives. The full Y-STR profile was obtained not only for reference sample LT but also for bone sample. All alleles agreed. To respect privacy of DNA profiles of living Toufar relatives, tangible alleles are not disclosed (Table 1).

Autosomal LR reached 60.9, increasing 50% prior probability to 98.38%, reaching thus Hummel's verbal equivalent of highly probable kinship. Y-STR haplotype of LT/JT? was not found in the database of 25,499 Haplotypes, yielding thus Y-STR LR = 19,069. As there was not known any possibility that another man of Toufar surname and lineage is buried in the pit, it was possible to

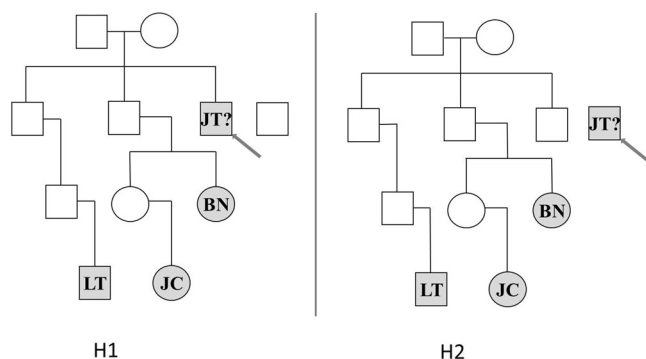


Fig. 6. Compared hypotheses.
H1: Josef Toufar is part of pedigree. H2: Josef Toufar is out of pedigree.

Table 1

Shared alleles between presumed Josef Toufar and his relatives are in grey.

	JC	BN	LT
D3S1358			
TH01			
D21S11			
D10S1248			
D1S1656			
D2S1338			
D16S539			
D22S1045			
vWA			
D8S1179			
FGA			
D2S441			
D12S391			
D19S433			
SE33			

combine A-STR and Y-STR LRs by multiplication. Combined LR reached 1,161,302 what would increase prior probability above legal limit for kinship practically proven (thus Josef Toufar identification practically proven) even for the priors as low as 1% (Table 2).

Identified remains of Josef Toufar were laid into silver coffin, sealed, and transferred to Čihošť. The funeral with a ceremonial Mass in the presence of Toufar family, Bishop of Diocese Hradec Králové Jan Vokál, Prague Archbishop Dominik Cardinal Duka, and several thousands of pilgrims was held there on 12 July 2015. Coffin was symbolically placed in the nave of Church of the Assumption of the Virgin Mary.

4. Discussion

Identification of skeletal remains by kinship is influenced by a prior pedigree knowledge, a degree of DNA degradation, and an availability of reference DNAs. In this paper, we identified the remains of Pater Josef Toufar from a mass grave in Prague-Ďáblice, using reference samples from niece, grand-niece, and grand-nephew and thigh bone sample from proband. First, we used PowerPlex ESX17 kit with 16 microsatellite loci plus amelogenin where *D18S51* was affected by locus dropout. Second, we used PowerPlex Y23 kit. Simulating the true Toufar relationship in Familias software with 15 loci (PowerPlex ESX17 minus locus *D18S51*), 27.9% of the 1,000 simulations with seed 1-2-3-4-5 were above the limit of LR = 400 that would increase 50% priors to more than 99.75% what is accepted by the Czech courts as kinship practically proven (median of simulated LR = 80, mean of simulated LR = 9,303). Thus, it was twice as probable that A-STR data would not be sufficient to yield legally defensible conclusion. Indeed, the obtained A-STR likelihood ratio (LR = 60.9, Table 3) had to be combined with Y-STR, in approach similar to one applied by others [11,12] to reach LR = 1,161,302, allowing the final conclusion.

While mtDNA is especially suitable for degraded DNA testing due to its circular nature and number of copies per cell [13], in this case its genotyping would not be informative due to pedigree constellation. Though there are currently available miniSTR genotyping kits with short amplicons [14] (i.e. AmpFISTR® MiniFiler™ PCR Amplification Kit, Applied Biosystems) that were successfully used even for older samples than ours [15], they have limited multiplexing level. Thus, we took a risk of not obtaining the usable data for all 16 PowerPlex ESX17 microsatellite loci. We

Table 2

Posterior probability of true Toufar identification given different priors and both A-STR and Y-STR data.

Prior P (%)	1	5	10	25	50	75
Posterior P (%)	99.991476	99.998364	99.999225	99.999742	99.999914	99.999971

Table 3

Posterior probability of true Toufar identification given different priors and A-STR data only.

P prior (%)	1	5	10	25	50	75
P posterior (%)	38.08	76.22	87.12	95.30	98.38	99.45

obtained heterozygotic genotypes for 11 loci, homozygotic (and potentially dropped out) profiles for 4 loci, and profile failure for 1 locus what compares favorably with 8 loci of the full MiniFiler kit.

Though two leading platforms of Massively Parallel Sequencing (MPS), IonTorrent [16] and MiSeq [17] have been successfully applied to kinship identification from degraded samples, we have not validated the method yet. So far, we reserve possibility to use MPS as complementary rather than replacing technique, in line with approach of Tasker et al. [18].

5. Conclusion

The case report of Josef Toufar remains identification confirms that it is possible to identify human skeletal remains older than 60 years buried in mass graves in the depth of 1.7 m, at duly operated Czech cemeteries when there is a social demand and an efficient collaboration is established among professions of geneticist, anthropologist, coroner, and archeologist.

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