Brazilian Journal of Forensic Sciences,
Medical Law and Bioethics

Journal homepage: www.ipebj.com.br/forensicjournal

Genetic Profiles of Y-Specific STR Markers Included in a Database Increase the Resolution of Sexual Crimes in the State of Goiás, Brazil

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Received 19 June 2023; Accepted 16 April 2024

Abstract. Considering that the vast majority of sex crimes are perpetrated by men, Y-STR molecular markers have become a valuable tool in criminal investigations. The Y-chromosome has shown promise in the identification of male DNA from samples, especially when this DNA occurs in small amounts or is mixed with genetic material of female origin, as it is commonly the case in vaginal swabs from rape exams. Given the high risk of recidivism among sexually violent offenders, the present study evaluates the effectiveness of an in-house Y-chromosome STR marker genetic profile database (BPG-YSTR) as a tool in the investigation of sex crimes in the state of Goiás, Brazil. We analyzed cases of sexual violence involving biological samples that tested positive and negative for spermatozoa, registered in the Superintendence of the Technical-Scientific Police of the state of Goiás. DNA extraction was performed by differential lysis using the *PrepFiler Express*™ (Applied Biosystems®) kit. DNA quantification was performed by Real-Time PCR, while amplification was performed with commercial multiplex AmpFlSTR ®Yfiler™ (AppliedBiosystems®), PowerPlex® Y23 (Promega) or

http://dx.doi.org/10.17063/bjfs12(2)y2024125-146

Yfiler™ Plus (AppliedBiosystems®) kits. Amplification products were separated by capillary electrophoresis in the ABI 3500 genetic analyzer®(AppliedBiosystems®). The inclusion of 595 haplotypic profiles in the BPG-YSTR identified genetic matches between biological evidence samples of sexual crimes involving 28 offenders and 126 victims. In 24.7% of the cases, the matches were identified exclusively by the BPG-YSTR, which means that analyses involving Y-chromosome markers added valuable information to the investigation of serial crimes in almost 1/4 of the cases. Therefore, Y-STR specific markers were shown to be a promising tool in the assistance of criminal investigations involving sexual crimes.

Keywords: Y-Chromosome; Sex crime; Y-STR profile database; Criminal investigation; Forensic genetics.

1. Introduction

Research involving the applicability of Y-chromosome molecular markers in human identification has advanced and gained notoriety in the support of criminal investigations of sexual violence, particularly in cases where haplotypic profiles are the only available evidence that could link the perpetrator to the crime[1-5].

Y-chromosome markers are not capable of individualizing a man in a population, since Y-specific loci are genetically linked and vertically transmitted in patrilineal lineages[6-8]. However, Y-chromosome marker profiles can assist police investigations by offering means to exonerate a suspect or help guide an investigation[6-9]. Y-specific markers have been useful in the identification of male sources of DNA in samples, especially when they occur in small quantities or are mixed with genetic material of female origin, as is typical in vaginal swabs from rape victims[9-13].

Genetic profile databases created for criminal prosecution are used to catalog genetic profiles derived from biological evidence and to automatically compare genetic data, identifying and reporting genetic matches[14,15]. The set of profiles deposited in a database becomes a tool that can aid criminal investigations when there are no immediate suspects, or when there is not enough information to initiate an investigation[5,6,16-20]. The use of these tools has helped police institutions and justice systems in the elucidation of old and highly complex cases[21,22].

Thus, Y-specific genetic profile databases should be used as auxiliary tools in the investigation of sexual assault cases due to their ability to identify serial crimes (making it possible to correlate different cases) when there is insufficient DNA to obtain autosomal profiles[5-23].

The current study seeks to assess the effectiveness of the in-house Y-specific genetic profile database of the Superintendence of the Technical-Scientific Police of the state of Goiás (BPG-YSTR) as an additional tool in the investigation of serial sexual crimes that took place in Goiás, Brazil.

2. Methods and materials

2.1 Study design and ethical aspects

The study was developed in accordance with the applicable norms of resolution nº 466/2012 of the National Health Council (Conselho Nacional de Saúde; CNS) and submitted and approved by the Research Ethics Committees of the Tropical Disease Hospital Dr. Anuar Auad (Hospital de Doenças Tropicais Dr. Anuar Auad; HDT/HAA) and of the Pontifical Catholic University of Goiás (Pontifícia Universidade Católica de Goiás; PUC/GO), under protocols nº 2,413,063 and 2,380,631, respectively. The study was conducted at the Laboratory of Biology and Forensic DNA of the Superintendence of the Technical-Scientific Police of the state of Goiás (Laboratório de Genética Forense da Superintendência da Polícia Técnico-Científica de Goiás; LBDF/SPTC-GO).

2.2 Sample selection

The study samples were derived from biological evidence of sex crimes (vulvar, vaginal, anal secretions, or biological material collected from skin bruises produced by suction) that occurred in the state of Goiás (Brazil), from 2004 to June 2020, related to police investigations involving female victims, stored at the Laboratory of Biology and Forensic DNA of the Superintendence of Forensic Scientific Police of the State of Goiás, Brazil. The absence of spermatozoa in the sample did not preclude its inclusion in the study.

2.3 Molecular analysis

DNA was extracted using the organic method with Amicon®Ultra (Merk), in accordance with the general regulations of the Brazilian Ministry of Justice [24], or by differential lysis using the PrepFiler Express™ Forensic DNA Extraction Kit on an AutoMate Express (Applied Biosystems®, California, USA), following the manufacturer's instructions.

DNA quantification was performed using the real-time PCR method. Y-chromosome molecular markers were amplified by PCR (Polymerase Chain Reaction), using multiplex Yfiler™ Plus (Applied Biosystems®, California, USA), PowerPlex® Y23 System (Promega®) or Yfiler® PCR Amplification (Applied Biosystems®, California, USA) kits. For the amplification of autosomal markers, we used multiplex Globalfiler ™ (Applied Biosystems®, California, USA) or PowerPlexFusion (Promega®) kits, following the manufacturer's instructions.

The amplification products were separated by capillary electrophoresis in the ABI 3130 ® or the ABI 3500® Genetic Analyzers (Applied Biosystems®, California, USA). The obtained genetic profiles were analyzed using the Gene Mapper ID-X software (Applied Biosystems®, California,USA).

The experiments performed in the validation tests for the PCR Amplification kits followed the SWGDAM guidelines and the results demonstrate the robustness and validity of the Yfiler™ Plus (Applied Biosystems®, California, USA)[13], Yfiler® PCR Amplification Kit (Applied Biosystems®, California, USA)[25] or PowerPlex® Y23 System (Promega®)[26] for use in forensic cases and database samples, accepted by the Managing Committee of the Integrated Network of Genetic Profile Databases[27].

2.4 Creation of the in-house Y-specific genetic profile database (BPG-YSTR) and automated searches

The BPG-YSTR was customized using Excel® and Access®, and in it were inserted 595 male genetic profiles based on biological evidence related to sexual crimes perpetrated against female victims, with 25 samples amplified with the Yfiler® PCR Amplification Kit (Applied Biosystems®, California, USA), 130 samples with the PowerPlex® Y23 System (Promega®), and 440 samples with the Yfiler™ Plus (Applied Biosystems®, California, USA). Thus, genetic profiles that had at least 17 haplotypic markers in common among the three amplification kits were inserted in the database. Additionally, as a quality control and safety measure, the haplotypic profiles of all members of the Laboratory of

Biology and Forensic DNA (LBDF/IC/SPTC-GO) team were included in the database to check for potential sample contamination during laboratory procedures.

Searches for genetic matches between samples were made after the inclusion of all haplotypic profiles in the BPG-YSTR. A genetic match is the similarity between Y-STR profiles derived from biological evidence of sexual crimes collected from different victims, allowing the evaluation of a perpetrator's recurrence.

The Y-STR haplotypic profiles involved in the present study were submitted to the YHRD Y-Chromosome Haplotype Reference Database (https://yhrd.org) and integrate the profile group submitted under Access Numbers YA004377 and YA004577.

2.5 Data analysis

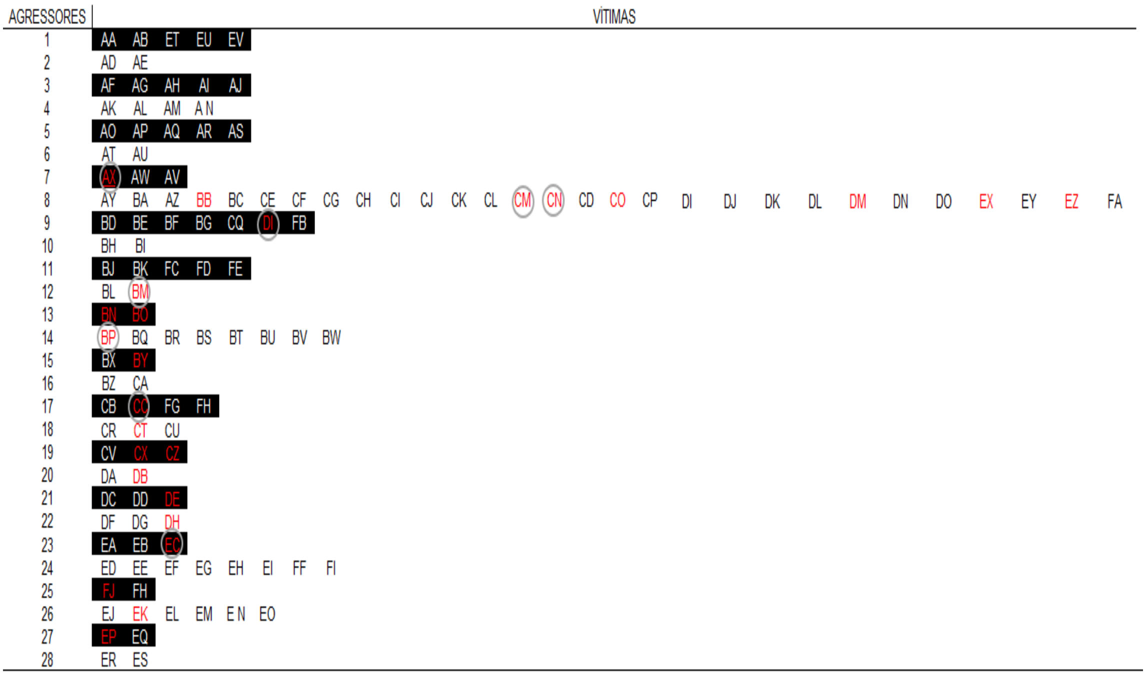
For the study objective, genetic matches reported by the BPG-YSTR were computed and compared with the results obtained with the Genetic Profile Database of the Superintendence of Technical-Scientific Police of the State of Goiás (BPG-SPTC/GO) using the CODIS 7 program. Study data were subjected to a descriptive analysis. The McNemar test was also used with a significance level of 5% and a degree of freedom of 1.

The BPG-YSTR haplotypic profile database is a tool independent from the Genetic Profile Database of the Superintendence of Technical-Scientific Police of the State of Goiás (BPG-SPTC/GO). While the BPG-YSTR searches for genetic matches among biological evidence analyzing Y-chromosome profiles, the BPG-SPTC/GO searches for genetic matches of the same evidence analyzing autosomal profiles.

3. Results and discussion

Among the 595 Y-STR haplotypes included in the BPG-YSTR, we identified genetic matches between evidence samples of sexually violent crimes involving 28 different serial offenders and 126 victims (Figure 1, Table 1). These matches demonstrate that a small number of offenders repeatedly commit acts of sexual violence against several victims, evidencing serial crimes. Table 2 illustrates matching haplotypic profiles reported by the BPG-YSTR. Each offender (known or unknown) was represented by a number, totaling 28 men; and each sample collected from the victim's body (vulvar secretion, vaginal, anal, or biological material collected from skin bruises produced by suction) was represented by letters to preserve the victim's identity.

Figure 1 shows the genetic matches identified by the BPG-YSTR. Each line represents a haplotypic profile present in biological evidence samples collected from different victims of sexual violence. Genetic matches between samples indicate serial offenders. For example, the profiles obtained from evidence related to victims AA, AB, ET, EU and EV are identical, indicating that a single offender was responsible for all five crimes.



AGRESSORES	VÍTIMAS
1	AA AB ET EU EV
2	AD AE
3	AF AG AH AI AJ
4	AK AL AM A N
5	AO AP AQ AR AS
6	AT AU
7	AX AW AV
8	AY BA AZ BB BC CE CF CG CH CI CJ CK CL CM CN CD CO CP DI DJ DK DL DM DN DO EX EY EZ FA
9	BD BE BF BG CQ DI FB
10	BH BI
11	BJ BK FC FD FE
12	BL BM
13	BN BO
14	BP BQ BR BS BT BU BV BW
15	BX BY
16	BZ CA
17	CB CC FG FH
18	CR CT CU
19	CV CX CZ
20	DA DB
21	DC DD DE
22	DF DG DH
23	EA EB EC
24	ED EE EF EG EH EI FF FI
25	FJ FH
26	EJ EK EL EM E N EO
27	EP EQ
28	ER ES

Figure 1 - Genetic matches reported by the BPG-YSTR involving 28 offenders and 126 victims. Each line represents a haplotypic profile derived from biological evidence (vaginal or anal secretions, or skin bruises produced by suction) collected from different victims of sexual violence. Cases highlighted in red correspond to the victims whose offenders were identified exclusively by the BPG-YSTR. Cases marked with circles were included in the BPG-SPTC/GO after being identified by the BPG-YSTR. Acronyms formed by meaningless alphabetic characters were used to preserve the identity of the victims.

Of the 595 cases evaluated by the present study, 101 were identified by the Genetic Profile Database of the Superintendence of the Technical-Scientific

Police of the state of Goiás[1] (BPG-SPTC/GO), while 126 were identified by the BPG-YSTR. Twenty-five of the reported cases presenting genetic coincidences (highlighted in red in Figure 1) were identified exclusively by the BPG-YSTR. This means that the indexation of Y-STR profiles increased the detection of serial sexual crimes by 24.7%.

The non-parametric statistical analysis using the McNemer Test did not show a statistically significant effect, which could be due to the study's design, with a single data collection performed in the analyzed time period and a low sample size. However, the results show the potential of genetic profile databases in elucidating rape crimes through the indication of alleged perpetrators and, consequently, the reduction of criminal recidivism, showing how valuable this tool can be to public safety and society[28-31].

Table 1. Serial sex crime cases reported by the BPG-YSTR.

Offender	Year of offense	Victim[2]	Y profiles matching between QSs	Autosomal profiles matching between QSs	Sample included in the BPG-YSTR	Sample included in the BPG-SPTC/GO
1	2013	AA	Yes	Yes	Yes	Yes
	2013	AB	Yes	Yes	Yes	Yes
	2013	ET	Yes	Yes	Yes	Yes
	2013	EU	Yes	Yes	Yes	Yes
	2013	EV	Yes	Yes	Yes	Yes
2	2011	AD	Yes	Yes	Yes	Yes
	2015	AE	Yes	Yes	Yes	Yes
3	2012	AF	Yes	Yes	Yes	Yes
	2013	AG	Yes	Yes	Yes	Yes
	2013	AH	Yes	Yes	Yes	Yes
	2013	AI	Yes	Yes	Yes	Yes
	2013	AJ	Yes	Yes	Yes	Yes
4	2015	AK	Yes	Yes	Yes	Yes
	2015	AL	Yes	Yes	Yes	Yes
	2015	AM	Yes	Yes	Yes	Yes
	2015	AN	Yes	Yes	Yes	Yes
5	2014	AO	Yes	Yes	Yes	Yes
	2014	AP	Yes	Yes	Yes	Yes
	2015	AQ	Yes	Yes	Yes	Yes
	2015	AR	Yes	Yes	Yes	Yes
	2015	AS	Yes	Yes	Yes	Yes

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[1] Official genetic profile database of the state of Goiás (Brazil), integrated into the national network of Genetic Profile Databases. It identifies genetic matches through the management (CODIS software) of autosomal markers.

Offender	Year of offense	Victim	Y profiles matching between QSs	Autosomal profiles matching between QSs	Sample included in the BPG-YSTR	Sample included in the BPG-SPTC/GO
6	2015	AT	Yes	Yes	Yes	Yes
	2015	AU	Yes	Yes	Yes	Yes
7	2015	AX	Yes	Yes	Yes	Yes, after the BPG-YSTR match
	2016	AW	Yes	Yes	Yes	Yes
	2016	AV	Yes	Yes	Yes	Yes
8	2008	AY	Yes	Yes	Yes	Yes
	2008	BA	Yes	Yes	Yes	Yes
	2008	AZ	Yes	Yes	Yes	Yes
	2009	BB	Yes	No autosomal profile obtained	Yes	No
	2009	BC	Yes	Yes	Yes	Yes
	2009	CE	Yes	Yes	Yes	Yes
	2009	CF	Yes	Yes	Yes	Yes
	2010	CG	Yes	Yes	Yes	Yes
	2010	CH	Yes	Yes	Yes	Yes
	2010	CI	Yes	Yes	Yes	Yes
	2011	CJ	Yes	Yes	Yes	Yes
	2014	CK	Yes	Yes	Yes	Yes
	2014	CL	Yes	Yes	Yes	Yes
	2015	CM	Yes	Yes	Yes	Yes, after the BPG-YSTR match
	2015	CN	Yes	Yes	Yes	Yes, after the BPG-YSTR match
	2016	CD	Yes	Yes	Yes	Yes
	2016	CO	Yes	No autosomal profile obtained	Yes	No
	2016	CP	Yes	Yes	Yes	Yes
	2016	DI	Yes	Yes	Yes	Yes
	2016	DJ	Yes	Yes	Yes	Yes
	2016	DK	Yes	Yes	Yes	Yes
	2016	DL	Yes	Yes	Yes	Yes
	2017	DM	Yes	No autosomal profile obtained	Yes	No
	2017	DN	Yes	Yes	Yes	Yes
	2017	DO	Yes	Yes	Yes	Yes
	2018	EX	Yes	No autosomal profile obtained	Yes	No
	2018	EY	Yes	Yes	Yes	Yes
8	2019	EZ	Yes	No autosomal profile obtained	Yes	No
	2019	FA	Yes	Yes	Yes	Yes
9	2014	BD	Yes	Yes	Yes	Yes
	2014	BE	Yes	Yes	Yes	Yes
	2014	BF	Yes	Yes	Yes	Yes
	2014	BG	Yes	Yes	Yes	Yes
	2014	CQ	Yes	Yes	Yes	Yes
	2014	DI	Yes	Yes	Yes	Yes, after the BPG-YSTR match
	2014	FB	Yes	Yes	Yes	Yes
10	2016	BH	Yes	Yes	Yes	Yes
	2016	BI	Yes	Yes	Yes	Yes
11	2013	BJ	Yes	Yes	Yes	Yes
	2014	BK	Yes	Yes	Yes	Yes
	2014	FC	Yes	Yes	Yes	Yes
	2015	FD	Yes	Yes	Yes	Yes
	2015	FE	Yes	Yes	Yes	Yes

Offender	Year of offense	Victim	Y profiles matching between QSs	Autosomal profiles matching between QSs	Sample included in the BPG-YSTR	Sample included in the BPG-SPTC/GO
12	2016	BL	Yes	Yes	Yes	Yes
	2017	BM	Yes	Yes	Yes	Yes, after the BPG-YSTR match
13	2011	BN	Yes	Yes	Yes	No
	2012	BO	Yes	Yes	Yes	No
14	2016	BP	Yes	Yes	Yes	Yes, after the BPG-YSTR match
	2016	BQ	Yes	Yes	Yes	Yes
	2016	BR	Yes	Yes	Yes	Yes
	2016	BS	Yes	Yes	Yes	Yes
	2016	BT	Yes	Yes	Yes	Yes
14	2016	BU	Yes	Yes	Yes	Yes
	2016	BV	Yes	Yes	Yes	Yes
	2016	BW	Yes	Yes	Yes	Yes
15	2019	BX	Yes	-	Yes	Yes
	2019	BY	Yes	No autosomal profile obtained	Yes	No
16	2016	BZ	Yes	Yes	Yes	Yes
	2016	CA	Yes	Yes	Yes	Yes
17	2014	CB	Yes	Yes	Yes	Yes
	2015	CC	Yes	Yes	Yes	Yes, after the BPG-YSTR match
	2014	FG	Yes	Yes	Yes	Yes
	2017	FH	Yes	Yes	Yes	Yes
18	2010	CR	Yes	Yes	Yes	Yes
	2011	CT	Yes	No autosomal profile obtained	Yes	No
	2012	CU	Yes	Yes	Yes	Yes
19	2012	CV	Yes	Yes	Yes	Yes
	2012	CX	Yes	No autosomal profile obtained	Yes	No
	2012	CZ	Yes	No autosomal profile obtained	Yes	No
20	2014	DA	Yes	Yes	Yes	Yes
	2014	DB	Yes	No autosomal profile obtained	Yes	No
21	2016	DC	Yes	Yes	Yes	Yes
	2016	DD	Yes	Yes	Yes	Yes
	2016	DE	Yes	No autosomal profile obtained	Yes	No
22	2016	DF	Yes	Yes	Yes	Yes
	2016	DG	Yes	Yes	Yes	Yes
	2016	DH	Yes	No autosomal profile obtained	Yes	No
23	2018	EA	Yes	Yes	Yes	Yes
	2018	EB	Yes	Yes	Yes	Yes
	2018	EC	Yes	Yes	Yes	Yes, after the BPG-YSTR match

Offender	Year of offense	Victim	Y profiles matching between QSs	Autosomal profiles matching between QSs	Sample included in the BPG-YSTR	Sample included in the BPG-SPTC/GO
24	2008	ED	Yes	Yes	Yes	Yes
	2009	EE	Yes	Yes	Yes	Yes
	2009	EF	Yes	Yes	Yes	Yes
	2009	EG	Yes	Yes	Yes	Yes
	2009	EH	Yes	Yes	Yes	Yes
	2012	EI	Yes	Yes	Yes	Yes
	2013	FF	Yes	Yes	Yes	Yes
	2017	FI	Yes	Yes	Yes	Yes
25	2013	FJ	Yes	No autosomal profile obtained	Yes	No
	2017	FH	Yes	Yes	Yes	Yes
	2016	EJ	Yes	Yes	Yes	Yes
	2016	EK	Yes	No autosomal profile obtained	Yes	No
26	2017	EL	Yes	Yes	Yes	Yes
	2018	EM	Yes	Yes	Yes	Yes
	2018	EN	Yes	Yes	Yes	Yes
	2019	EO	Yes	Yes	Yes	Yes
27	2015	EP	Yes	No autosomal profile obtained	Yes	No
	2013	EQ	Yes	Yes	Yes	Yes
28	2017	ER	Yes	Yes	Yes	Yes
	2018	ES	Yes	Yes	Yes	Yes

QSs: questioned samples .
BPG-YSTR: in-house Y-chromosome genetic profile database.
BPG-SPTC/GO: Genetic Profile Database of the Superintendence of the Technical-Scientific Police of the state of Goiás, Brazil. As shown in Figure 1, cases highlighted in red correspond to victims reported exclusively by the BPG-YSTR. Cases marked with circles generated autosomal genetic profiles which were included in the BPG-SPTC/GO after a match was identified by the Y-STR database.

Including Y-STR profile data in these investigations has improved the system's ability to link multiple victims of sexual violence to a specific offender, enhancing the effectiveness of the BPG-SPTC/GO database. For the 25 aforementioned samples, only autosomal profiles that failed to meet the criteria for inclusion in the BPG-SPTC/GO or Y-STR profiles could be obtained due to the small amount of male DNA contained in them. Thus, the use of Y-STR markers deserves attention when the goal is to identify the male contributor in these samples, such as vaginal smears from rape cases, where isolating the autosome DNA of the perpetrator is challenging due to the minimal quantity present and the mixing with genetic material of female origin[6,9,32-35].

Table 2. Matching haplotypic profiles reported by the BPG-YSTR.

Offender	Victim	PCR Kit	DYS389I	DYS448	DYS389II	DYS19	DYS391	DYS438	DYS437	DYS635	DYS390	DYS439	DYS392	DYS393	DYS458	DYS385	YGATAH4	DYS456	DYS576	DYS481	DYS533	DYS570	DYS627	DYS460	DYF387S1	DYS518	DYS449
1	AA	Yfiler	13	19	28	14	11	12	15	23	24	12	13	14	17	11-13	12	16									
	AB	Y23	13	19	28	14	11	12	15	23	24	12	13	14	17	11-13	12	16	18	22	12	17					
	ET	Y23	13	19	28	14	11	12	15	23	24	12	13	14	17	11-13	12	NA	18	22	12	17					
	EU	Y23	13	19	28	14	11	12	15	23	24	12	13	14	17	11-13	12	NA	18	22	12	17					
	EV	Y23	13	19	28	14	11	12	15	23	24	12	13	14	17	11-13	12	NA	18	22	12	17					
2	AD	Y23	14	21	30	15	10	11	14	23	21	12	11	14	18	16-18	10	16	16	26	12	17					
	AE	Y23	14	21	30	15	10	11	14	23	21	12	11	14	18	16-18	10	16	16	26	12	17					
3	AF	Yfiler	14	19	30	14	10	9	14	21	24	12	11	13	17	13-15	11	15									
	AG	Yfiler	14	19	30	14	10	9	14	21	24	12	11	13	17	13-15	11	15									
	AH	Yfiler	14	19	30	14	10	9	14	21	24	12	11	13	17	13-15	11	15									
	AI	Yfiler	14	19	30	14	10	9	14	21	24	12	11	13	17	13-15	11	15									
	AJ	Yfiler	14	19	30	14	10	9	14	21	24	12	11	13	17	13-15	11	15									
4	AK	Y23	14	19	30	14	11	10	15	23	22	12	13	13	17	10-14	12	15	18	22	12	17					
	AL	Y23	14	19	30	14	11	10	15	23	22	12	13	13	17	10-14	12	15	18	22	12	17					
	AM	Y23	14	19	30	14	11	10	15	23	22	12	13	13	17	10-14	12	15	18	22	12	17					
	A N	Yfiler	14	19	30	14	11	10	15	23	22	12	13	13	17	10-14	12	15									
5	AO	Y23	13	21	31	15	10	11	14	21	21	12	10	13	17	16-18	12	15	17	27	11	21					
	AP	Y23	13	21	31	15	10	11	14	21	21	12	10	13	17	16-18	12	15	17	27	11	21					
	AQ	Y23	13	21	31	15	10	11	14	21	21	12	10	13	17	16-18	12	15	17	27	11	21					
	AR	Y23	13	21	31	15	10	11	14	21	21	12	10	13	17	16-18	12	15	17	27	11	21					
	AS	Y23	13	21	31	15	10	11	14	21	21	12	10	13	17	16-18	12	15	17	27	11	21					
6	AT	Y23	13	18	30	15	11	12	14	23	24	12	13	13	18	11-13	12	16	20	23	11	17					
	AU	Y23	13	18	30	15	11	12	14	23	24	12	13	13	18	11-13	12	16	20	23	11	17					
7	AX	Y23	13	18	29	15	10	11	15	25	24	12	13	13	15	12-13	12	15	17	24	13	16					
	AW	Yfilerplus	13	18	29	15	10	11	15	25	24	12	13	13	15	12-13	12	15	17	24	13	16	19	10	35-38	37	27
	AV	Y23	13	18	29	15	10	11	15	25	24	12	13	13	15	12-13	12	15	17	24	13	16					
8	AY	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	NA					
	BA	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	AZ	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	BB	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	BC	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	CE	Yfilerplus	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20	19	10	36-38	37	36
	CF	Yfilerplus	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20	19	10	36-38	37	36
	CG	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	CH	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	CI	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	CJ	Y23	13	19	30	13	10	9	14	22	23	12		13	15	17-19	12	16	18	22	12	20					
	CK	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	CL	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	CM	Yfilerplus	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20	19	10	36-38	37	36
	CN	Y23	13	19	30	13	10	9	14	22	23		11	13	15	17-19	12	16	18	22	12	20					
	CD	Yfilerplus	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20	19	10	36-38	37	36
	CO	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	CP	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	DI	Yfilerplus	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20	19	10	36-38	37	36
	DJ	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	DK	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	DL	Yfilerplus	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20	19	10	36-38	37	36
	DM	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	DN	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	DO	Yfilerplus	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20	19	10	36-38	37	36
	EX	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					
	EY	Y23	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20					

Offender	Victim	PCR Kit	DYS389I	DYS448	DYS389II	DYS19	DYS391	DYS438	DYS437	DYS635	DYS390	DYS439	DYS392	DYS393	DYS458	DYS385	YGATAH4	DYS456	DYS576	DYS481	DYS533	DYS570	DYS627	DYS460	DYF387S1	DYS518	DYS449
	EZ	Yfilerplus	13	19	30	13	10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	12	20	19	10	36-38	37	36
	FA	Y23	13	19	30		10	9	14	22	23	12	11	13	15	17-19	12	16	18	22	NA	20					
9	BD	Y23	13	20	31	13	11	11	15	23	25	12	14	13	17	14-14	13	15	17	25	11	15					
	BE	Yfilerplus	13	20	31	13	11	11	15	23	25	12	14	13	17	14-14	13	15	17	25	11	15	18	10	35-38	40	29
	BF	Yfilerplus	13	20	31	13	11	11	15	23	25	12	14	13	17	14-14	13	15	17	25	11	15	18	10	35-38	40	29
	BG	Y23	13	20	31	13	11	11	15	23	25	12	14	13	17	14-14	13	15	17	25	11	15					
	CQ	Y23	13	20	31	13	11	11	15	NA	25	12	14	13	17	14-14	13	15	17	25	11	15					
	DI	Yfilerplus	13	20	31	13	11	11	15	23	25	12	14	13	17	14-14	NA	15	17	25	11	15	18	10	35-38	NA	29
	FB	Y23	13	20	31	13	11	11	15	23	25	12	14	13	17	14-14	13	15	17	25	11	15					
10	BH	Yfilerplus	13	19	29	14	11	12	15	23	23	NA	12	13	16	12-13	12	17	19	24	12	18	23	10	35-37	39	30
	BI	Yfilerplus	13	19	29	14	11	12	15	23	23	NA	12	13	16	12-13	12	17	19	24	12	18	23	10	35-37	39	30
11	BJ	Y23	14	20	30	13	9	10	14	21	24	10	11	13	18	13-14	11	16	18	27	11	22					
	BK	Y23	14	20	30	13	9	10	14	21	24	10	11	13	18	13-14	11	16	18	27	11	22					
	FC	Y23	14	20	30	13	9	10	14	21	24	10	11	13	18	13-14	11	16	18	27	11	22					
	FD	Yfilerplus	14	20	30	13	9	10	14	21	24	10	11	13	18	13-14	11	16	18	27	11	22	18	11	37-37	43	33
	FE	Yfilerplus	14	20	30	13	9	10	14	21	24	10	11	13	18	13-14	11	16	18	27	11	22	18	11	37-37	43	33
12	BL	Yfilerplus	12	20	30	14	10	10	17	19	22	12	11	13	14	15-15	11	15	16	24	12	18	18	11	37-38	35	37
	BM	Y23	12	20	30	14	10	10	17	19	22	12	11	13	14	15-15	11	15	16	24	12	18					
13	BN	Yfiler	14	20	30	13	9	10	14	21	23	10	11	13	17	13-14	12	15									
	BO	Yfiler	14	20	30	13	9	10	14	21	23	10	11	13	17	13-14	12	15									
14	BP	Y23	13	21	30	15	11	10	16	21	22	12	10	13	18	14-15	11	15	14	21	10	20					
	BQ	Y23	13	21	30	15	11	10	16	21	22	12	10	13	18	14-15	11	15	14	21	10	20					
	BR	Yfilerplus	13	21	30	15	11	10	16	21	22	12	10	13	18	14-15	11	15	14	21	10	20	19	11	36-39	40	33
	BS	Yfilerplus	13	21	30	15	11	10	16	21	22	12	10	13	18	14-15	11	15	14	21	10	20	19	11	36-39	40	33
	BT	Yfilerplus	13	21	30	15	11	10	16	21	22	12	10	13	18	14-15	11	15	14	21	10	20	19	11	36-39	40	33
	BU	Yfilerplus	13	21	30	15	11	10	16	21	22	12	10	13	18	14-15	11	15	14	21	10	20	19	11	36-39	40	33
	BV	Yfilerplus	13	21	30	15	11	10	16	21	22	12	10	13	18	14-15	11	15	14	21	10	20	19	11	36-39	40	33
	BW	Yfilerplus	13	21	30	15	11	10	16	21	22	12	10	13	18	14-15	11	15	14	21	10	20	19	11	36-39	40	33
15	BX	Y23	19	14	19	30	14	11	21	NA	13	12	15	16	23	24	14	14	NA	13	12	11-15					
	BY	Y23	19	14	19	30	14	11	21	NA	13	12	15	16	23	24	14	14	NA	13	12	11-15					
16	BZ	Yfilerplus	NA	20	NA	13	10	11	NA	NA	24	NA	14	13	19	14-18	11	16	17	23	13	17	18	10	37-39	38	32
	CA	Yfilerplus	NA	20	NA	13	10	11	NA	NA	24	NA	14	13	19	14-18	11	16	17	23	13	17	18	10	37-39	38	32
17	CB	Yfilerplus	13	22	31	15	10	12	14	21	21	12	11	13	15	16-16	12	15	15	28	11	19	19	10	35-39	38	29
	CC	Y23	13	22	31	15	10	12	14	21	21	12	11	13	15	16-16	12	15	15	28	11	19					
	FG	Y23	13	22	31	15	10	12	14	21	21	12	11	13	15	16-16	12	15	15	28	11	19					
	FH	Y23	13	22	31	15	10	12	14	21	21	12	11	13	15	16-16	12	15	15	28	11	19					
18	CR	Y23	14	18	31	14	11	12	14	23	24	12	13	13	17	11-11	11	15	17	22	12	17					
	CT	Y23	14	18	31	14	11	12	14	23	24	12	13	13	17	11-11	11	15	17	22	12	17					
	CU	Y23	14	18	31	14	11	12	14	23	24	12	13	13	17	11-11	11	15	17	22	12	17					
19	CV	Y23	12	19	29	15	10	9	16	22	25	11	11	12	16	13-18	11	13	17	23	12	18					
	CX	Y23	12	19	29	15	NA	9	16		25	11	11	12	16	13-18	11	13	17	23	12	18					
	CZ	Y23	12	19	29	15	10	9	16	22	25	11	11	12	16	13-18	11	13	17	23	12	18					
20	DA	Y23	13	21	31	15	11	11	14	21	21	11	11	13	17	16-18	12	15	15	28	11	20					
	DB	Y23	13	21	31	15	11	11	14	21	21	11	11	13	17	16-18	12	15	15	28	11	20					
21	DC	Y23	13	18	29	14	10	12	15	23	24	13	13	13	17	41974	12	14	19	22	12	18					
	DD	Y23	13	18	29	14	10	12	15	23	24	13	13	13	17	41974	12	14	19	22	12	18					
	DE	Y23	13	18	29	14	10	12	15	23	24	13	13	13	17	41974	12	14	19	22	12	18					
22	DF	Yfilerplus	13	20	30	13	10	10	14	23	24	13	11	13	15	16-17	12	16	17	22	12	18	21	10	36-37	40	32
	DG	Y23	13	20	30	13	10	10	14	23	24	13	11	13	15	16-17	12	16	17	22	12	18					
	DH	Y23	13	20	30	NA	10	10	14	23	24	13	11	13	15	16-17	12	16	17	22	12	18					
23	EA	Y23	12	19	28	16	10	9	16	22	24	11	11	13	16	14-17	11	12		24	12	16					
	EB	Y23	12	19	28	16	10	9	16	22	24	11	11	13	16	14-17	11	12	16	24	12	16					
	EC	Yfilerplus	12	19	28	16	10	9	16	22	24	11	11	13	16	14-17	11	12	16	24	12	16	21	11	37-38	38	29
24	ED	Y23	13	21	29	15	9	9	14	21	23	12	11	12	14	13-16	12	17	16	22	12	18					
	EE	Y23	13	21	29	15	9	9	14	21	23	12	11	12	14	13-16	12	17	16	22	12	18					
	EF	Y23	13	21	29	15	9	9	14	21	23	12	11	12	14	13-16	12	NA	16	22	12	18					

	EG	Y23	13	21	29	15	9	9	14	21	23	12	11	12	14	13-16	12	17	16	22	12	18					
	EH	Yfiler	13	21	29	15	9	9	14	21	23	12	11	12	14	13-16	12	17									
	EI	Y23	13	21	29	15	9	9	14	21	23	12	11	12	14	13-16	12	17	16	22	12	18					
	FF	Y23	13	21	29	15	9	9	14	21	23	12	11	12	14	13-16	12	17	16	22	12	18					
	FI	Y23	13	21	29	15	9	9	14	21	23	12	11	12	14	13-16	12	17	16	22	12	18					
25	FJ	Y23	13	19	29	14	10	12	15	23	24	11	13	13	20	11-14	12	16	16	22	12	18					
	FH	Y23	13	19	29	14	10	12	15	23	24	11	13	13	20	11-14	12	16	16	22	12	18					
26	EJ	Yfilerplus	13	20	29	14	10	12	15	23	24	11	13	13	17	11-14	12	15	19	22	12	17	21	10	34-36	37	29
	EK	Y23	13	20	29	14	10	12	15	23	24	11	13	13	17	11-14	12	15	19	22	12	17					
	EL	Y23	13	20	29	14	10	NA	15	23	24	11	13	13	17	11-14	12	15	19	22	12	17					
	EM	Yfilerplus	13	20	29	14	10	12	15	23	24	11	13	13	17	11-14	12	15	19	22	12	17	21	10	34-36	37	29
	E N	Yfilerplus	13	20	29	14	10	12	15	23	24	11	13	13	17	11-14	12	15	19	22	12	17	21	10	34-36	37	29
	EO	Y23	13	20	29	14	10	12	15	23	24	11	13	13	17	11-14	12	15	19	22	12	17					
27	EP	Yfilerplus	13	19	29	15	11	12	15	23	24	12	13	13	16	11-14	12	15	18	22	12	17	22	12	35-37	40	29
	EQ	Y23	13	19	29	15	11	12	15	23	24	12	13	13	16	11-14	12	15	18	22	12	17					
28	ER	Y23	14	20	31	14	9	9	14	21	24	11	11	13	16	16-17	12	14	16	23	11	18					
	ES	Y23	14	20	31	14	9	9	14	21	24	11	11	13	16	16-17	12	14	16	23	11	18					

NA: non-amplified marker

The case of serial offender number 8 (Figure 1), investigated by Operation Impus, conducted by the police force of the state of Goiás, exemplifies the usefulness of Y-STR profile data. Eight of the victims of this offender were linked to him through the Y-STR profile obtained from their vaginal secretions. The haplotype match was added to the body of evidence of this investigation, resulting in the arrest of one of the most active serial rapists of Brazil, in 2019 [28]. Thus, the use of genetic profile databases greatly contributes to justice by confirming the authorship of particular crimes and discovering serial criminals[36-38].

The BPG-YSTR can also act as a screening tool for genetic matches, as exemplified in cases marked with a circle in Figure 1 and Table 1. Although the biological evidence originating from the offender (8 samples in total) did present Y-STR and autosomal profiles, they failed to meet the requirements for inclusion in the BPG-SPTC/GO, for reasons such as the absence of a reference sample from a consenting partner, or that they presented a minority autosomal profile with low RFU signal due to possible dropout. After the identification of haplotypic matches between different biological samples by the BPG-YSTR, these doubts were resolved. This allowed for the inclusion of the profiles in the BPG-SPTC/GO, and a report containing statistical analysis of autosomal results was issued to inform the police authorities of the genetic match.

Thus, the use of Y-chromosome genetic markers provided information—until then unlisted in the official database—that assisted eight police investigations. Autosomal profiles corroborated the results provided by the Y-STR markers.

Similar to this research, a study conducted by Neuhuber et al. [5], in Austria, demonstrated the relevance of DNA examination involving Y-chromosome analyses in sex crimes. In 38 of 239 analyzed rape cases, Y-STR profiles collected at crime scenes were able to elucidate serial crimes and assist in the investigation of potential perpetrators. Based on those findings, the Austrian National DNA Database was expanded to include Y-chromosome markers. The potential of a forensic Y-STR database to effectively work with a small number of haplotypic profiles was also demonstrated then, and the authors concluded that the database should yield even more results and help elucidate more serial crimes as it received more entries. They also stressed that results based on such data should be interpreted sensibly, because of the hereditary transmission of Y-chromosome markers.

In another study, conducted by Purps et al. [34], samples of 287 criminal cases of sexual violence were examined. Among them, 133 complete haplotypic profiles were obtained, increasing by 21% the number of informative profiles when compared to the results obtained exclusively by autosomal markers. According to this research, if the samples had not been submitted to a Y-STR analysis, 10% of cases would be inconclusive.

In the present study, the BPG-YSTR contributed to criminal investigations in two ways: by (1) linking victims of sexual violence to specific offenders based on the similarity between haplotypic profiles acquired from biological evidence, providing authorities with more resources to initiate investigations and prosecute cases; and by (2) acting as a screening tool for genetic matches for subsequent inclusion in the BPG-SPTC/GO.

We also estimate that results based on Y-STR data could become more relevant once more samples that tested negative for spermatozoa are inserted in the database[5,39]. In these situations, matches obtained by the BPG-YSTR can help to start an investigation by connecting several victims to a single offender. The investigation can then move forward based on different evidence, such as the *modus operandi* of the offender, the region where the crime was committed, the offender's physical description, descriptions of vehicles used and eye-witness accounts. The fact that men belonging to the same patrilineal lineage share the same haplotypic profile should always be considered in the interpretation of Y-STR matching results, given the direct, non-recombinant transmission of Y-chromosome genes[7,8,40-43]. However, even taking heredity into account, this tool considerably narrows the number of possible men involved in a crime.

Negative results in a spermatozoa analysis can hinder an investigation, weakening the hypothesis of sexual intercourse. However, it is possible to obtain genetic profiles using Y-STR markers of nucleated cells present in seminal fluid, such as leukocytes and immature germ cells. Y-STR markers become promising genetic tools when the analysis of autosomal markers is inconclusive, such as in sexual assaults perpetrated by offenders that are vasectomized, azoospermic or when there was no ejaculation[5,34]. Thus, genetic tests involving y-chromosome markers can provide material evidence for criminal investigations due, particularly, to their ability to detect traces of male DNA in samples dominated by the victim's DNA, a situation commonly found in sex crimes[6,32-35].

Furthermore, in genetic mixtures where the proportion of female DNA is considerably higher than male DNA, allelic dropout of the minor contributor may occur, a phenomenon called preferential amplification of the major contributor. This is a typical situation found in samples from vaginal swabs from rape cases[6,35]. Studies have shown that DNA from the minor contributor (offender) is usually undetectable when the proportions of autosomal DNA are equal or less than 1:50[44,45]. Y-STR profiles, on the other hand, can be obtained in mixtures of male:female DNA with a ratio of 1:1,000. That is, it is possible to obtain satisfactory results using Y-STR markers even when a sample contains 1,000 times more DNA of female origin[5,13,46].

In a study led by De Paula[39], the authors observed that Y-chromosome markers allowed the detection of male DNA in 43% of samples from sex crimes that tested negative for spermatozoa. This finding led to changes in the laboratory procedures at the Forensic DNA Research Institute of the Civil Police of the Federal District, Brazil (IPDNA/DF). The IPDNA/DF now submits these samples to Y-STR genetic analysis in hope that they will assist in the identification of rapists.

Based on the data presented, the Laboratory of Biology and Forensic DNA of the Superintendence of the Technical-Scientific Police of the state of Goiás now utilizes Y-chromosome markers to examine DNA in all cases of sexual crime, including those containing samples which tested negative for spermatozoa. Considering the nationwide backlog of unprocessed samples related to sexual crimes and the high prevalence of sexual crime samples that test negative for spermatozoa and PSA screening exams, obtaining Y-STR profiles for these samples has become essential[2,47,48]. In these cases, in particular, Y-STR databases will help manage haplotypic profiles and add important information for the resolution of crimes by identifying genetic matches, the same way it happened in Austria[5].

Having genetic evidence in hand that links different victims of sexual crimes can help authorities to initiate an investigation and build a body of evidence to support the prosecution and punishment of a crime. Considering the high cost of DNA testing, we cannot overlook the potential of this tool in helping to solve sex crimes[19,20,2931,49,50]. The improvement of statistical interpretation methods also make Y-STR markers more useful every day[47,48].

The investigation of sex crime cases using forensic genetics becomes difficult when male DNA from the offender is mixed with high concentrations of female DNA from the victim. The complexity of a deconvolution of mixtures and the subsequent lack of a reliable autosomal profile of the perpetrator highlight the need for alternative tools to elucidate these crimes. Therefore, research involving Y-chromosome DNA genetic profiles and their application in the investigation of violent sexual crimes is very important[5,6,9,32-35,39].

4. Conclusions

The use of the BPG-YSTR showed promise in the elucidation of sexual crimes in cases where there is biological evidence containing the autosomal genotypes of offenders, but where the samples do not meet the admissibility requirements for inclusion in the genetic profile database of the Superintendence of the Technical-Scientific Police of Goiás. Thus, the Y-STR profile database can act as a tool for linking serial crime cases perpetrated by a potential offender. The authorship of a crime can be determined by analyzing autosomal results.

Our research shows that in 24.7% of evaluated cases of sexual violence genetic matches were identified exclusively by the BPG-YSTR, which means that analyses involving Y-chromosome markers added valuable information to the investigation of serial crimes in almost 1/4 of the cases. These results show that BPG-YSTR data was valuable for the elucidation of sexual crimes, especially in cases where autosomal profiles were not obtained. The Y-STR profile database can act as a tool for linking serial crime cases perpetrated by a potential offender. Even when genetic information contained in the Y-chromosome cannot, by itself, identify the perpetrator of a crime, it can considerably reduce the number of suspects in an investigation. Furthermore, linking multiple victims to a single offender can establish a cohesive body of evidence that allows an investigation to start and facilitates prosecution.

Therefore, public policies should consider investing in forensic laboratories in order to secure resources for obtaining material evidence. It is imperative to invest in technologies and new genetic tools, such as the BPG-YSTR, to elucidate crimes and identify potential threats to society, reducing the likelihood of recidivism.

Acknowledgements

This study was supported by the Superintendence of the Technical and Scientific Police of the state of Goiás (SPTC-GO), the Department of Health of the State of Goiás (SES/GO) and the Laboratory of Human Cytogenetics and Molecular Genetics (LaGene). Arão, GOL was sponsored by a scholarship from the Foundation for Research Support of the State of Goiás - FAPEG (201710267000590) We also thank the support of CAPES (Coordination for the improvement of Higher Education Personnel).

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