

3-2-2015

# Mitochondrial DNA Suggests a Western Eurasian origin for Ancient (Proto-) Bulgarians

D V. Nesheva

*Department of Medical Genetics, Medical University of Sofia*

S Karachanak-Yankova

*Department of Medical Genetics, Medical University of Sofia*

M Lari

*Department of Evolutionary Biology, Laboratory of Anthropology, Molecular Anthropology/Paleogenetics Unit, University of Florence, Florence, Italy*

Y Yordanov

*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences*

A Galabov

*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences*

*See next page for additional authors*

---

## Recommended Citation

Nesheva, D V.; Karachanak-Yankova, S; Lari, M; Yordanov, Y; Galabov, A; Caramelli, David; and Toncheva, Draga, "Mitochondrial DNA Suggests a Western Eurasian origin for Ancient (Proto-) Bulgarians" (2015). *Human Biology Open Access Pre-Prints*. Paper 69. [http://digitalcommons.wayne.edu/humbiol\\_preprints/69](http://digitalcommons.wayne.edu/humbiol_preprints/69)

This Open Access Preprint is brought to you for free and open access by the WSU Press at DigitalCommons@WayneState. It has been accepted for inclusion in Human Biology Open Access Pre-Prints by an authorized administrator of DigitalCommons@WayneState.

---

**Authors**

D V. Nesheva, S Karachanak-Yankova, M Lari, Y Yordanov, A Galabov, David Caramelli, and Draga Toncheva

# Mitochondrial DNA Suggests a Western Eurasian origin for Ancient (Proto-) Bulgarians

D. V. Nesheva,<sup>1</sup> S. Karachanak-Yankova,<sup>1</sup> M. Lari,<sup>2</sup> Y. Yordanov,<sup>3</sup> A. Galabov,<sup>4</sup> D. Caramelli,<sup>2\*†</sup> and D. Toncheva<sup>1\*†</sup>

<sup>1</sup>Department of Medical Genetics, Medical University of Sofia, Sofia, Bulgaria.

<sup>2</sup>Department of Biology, Laboratory of Anthropology, Molecular Anthropology/Paleogenetics Unit, University of Florence, Florence, Italy.

<sup>3</sup>Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria.

<sup>4</sup>The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria.

\*These authors have equally contributed to this work.

†Correspondence to: Draga Toncheva, Department of Medical Genetics, Medical Faculty, Medical University, Sofia 2 Zdrave str. E-mail: dragatoncheva@gmail.com. David Caramelli, Department of Biology, Laboratory of Anthropology, Molecular Anthropology/Paleogenetics Unit, University of Florence, Florence, Italy. E-mail: david.caramelli@unifi.it.

**Key Words: MITOCHONDRIAL DNA, ANCIENT DNA, HAPLOTYPE, HAPLOGROUP, PROTO-BULGARIANS.**

## **Abstract**

Ancient (proto-) Bulgarians have long been thought to as a Turkic population. However, evidence found in the past three decades show that this is not the case. Until now, this evidence does not include ancient mitochondrial DNA (mtDNA) analysis. In order to fill this void, we have collected human remains from the VIII-X century AD located in three necropolises in Bulgaria: Nojarevo (Silistra region) and Monastery of Mostich (Shumen region), both in Northeast Bulgaria and Tuhovishte (Satovcha region) in Southwest Bulgaria. The phylogenetic analysis of 13 ancient DNA samples (extracted from teeth) identified 12 independent haplotypes, which we further classified into mtDNA haplogroups found in present-day European and Western Eurasian populations. Our results suggest a Western Eurasian matrilineal origin for proto-Bulgarians as well as a genetic similarity between proto- and modern Bulgarians. Our future work will provide additional data which will further clarify proto-Bulgarian origins; thereby adding new clues to current understanding of European genetic evolution.

The development of anthropogenetics and paleogenetics and the increase of their role in evolutionary science have given rise to a new scientific field of ancient DNA research.

Technological improvements now allow the retrieval of mtDNA from museum specimens, archaeological finds, and fossil remains. By sequencing mitochondrial hypervariable fragments of ancient DNA extracted from skeletal remains, researchers can classify mtDNA into maternal lines according to their sequence polymorphisms, thereby creating chronologies that link contemporary humans with their ancestors (Adachia et al. 2004; Forster 2004; Yao and Zhang

2000; Yonggang and Yaping 2003). In this study, we used ancient mtDNA to investigate the origins of proto-Bulgarians.

Many studies have focused on the origins of ancient (proto-) Bulgarians. This interest is most likely related to the fact that Danubian Bulgaria, the proto-Bulgarian state created in the seventh century AD is the only ancient state in Europe that has retained its name to the present day. The ancient Bulgarian state was officially recognized by the Eastern Roman Empire (Byzantium) in 681, after Kan Asparuh led its army to victory over the 80 000 army of the Eastern Roman Empire (Byzantium) in 680. At that time Bulgaria extended to the Balkan Mountains.

One concept of the origin of proto-Bulgarians defines them as a Turkic population, mostly referred to as Hun-Tatars (Huns Mongols). Tatars are the Russian designation for the Mongols—descendants of Genghis Khan, who invaded Russia in the thirteenth century. This hypothesis was first presented in *Dějiny národa bulharského* (History of the Bulgarians) by a Czech historian, diplomat, and Slavicist, who worked as a politician in Bulgaria from 1879 to 1884 (see Jireček 1878 and Jireček 1876 for English and German translations). This idea was followed by a prominent Bulgarian medievalist (Slatarski 1918; Zlatarski 1914, 1918, 1970), and still has followers to this day; some of whom claim that the small Bulgarian horde was “submerged” in the Slavic demic sea.

However, researchers such as Peter Koledarov, Peter Dobrev, and Georgi Bakalov reject the idea of a Hun-Tatar (Turkic) origin for proto-Bulgarians (Dobrev 1991, 1998, 2005; Fol et al. 2000), and recently the number of those who agree with them has been increasing (e.g., Daskalov 2011; Haefs 2009; Stamatov 1997). Their rejection of a Hun-Tatar origin is based on archeo-anthropological, historical, linguistic, and ethnographic evidence, which has been increasing over the past three decades.

Such research has shown that, following the second century, proto-Bulgarians created three countries in Europe: Danubian Bulgaria, Volga-Kama Bulgaria, and Old Great Bulgaria in northern Caucasus. They also built town-fortresses, organized powerful armies, and developed civilizations, economies, and art. Leading Turkologists have also presented evidence that the language of proto-Bulgarians does not reflect the Turkic linguistic family; instead it gravitates toward the Pamir languages of the East Iranian group, which belong to the Indo-European branch of languages (Bazin 1974; Manchen-Helfen 1973; Menges 1968; Pritsak 1955). Furthermore, writings from ancient Greek, old German, old Khazar, and Proto-Bulgarian authors suggest that proto-Bulgarians were a numerous people (Beshevliev 1993; Daskalov 2011; Dujchev 1963; Petrov and Gjuzelev 1979), comprising 32–60% of the population of Danubian Bulgaria (Dimitrov 2005; Rashev 1993). As history lacks examples of advanced, developed populations, such as proto-Bulgarians, being assimilated by tribes that are at an early stage of social development, like the Balkan Slavic tribes, it is unlikely that proto-Bulgarians were subsumed by such a group.

To date, analysis of ancient mtDNA from remains found in Bulgaria is missing from the literature. Thus, we report the first data from mitochondrial phylogenetic analysis of ancient DNA retrieved from human remains found in Bulgarian lands. We describe the mtDNA composition found in our samples and discuss the obtained data from genetic, anthropological and historical point of view in order to unravel the origin of ancient proto-Bulgarian populations.

## **Materials and Methods**

In order to minimize possible founder effects, we have analyzed human skeletal remains found in different Bulgarian lands and dating to different periods of the first Bulgarian state - Danubian

Bulgaria (VIII-X century AD). The Danubian Bulgaria population consisted primarily of proto-Bulgarian and Slavic tribes who occupied areas inhabited in antiquity by Thracian populations. The proto-Bulgarians practiced typical burial traditions, whereas the Slavs practiced cremation (Jordanov and Timeva 2010; Rashev 2008; Rashev et al. 1986, 1987, 1988, 1989). Based on this and on historical and anthropological data, the analyzed remains are considered as proto-Bulgarian.

Specimens (teeth) were collected from graves in three necropolises: the Monastery of Mostich (Shumen region) and Nojarevo (Silistra region) in Northeast Bulgaria; Tuhovishte (Satovcha region) in Southwest Bulgaria (Figure 1). Table 1 provides descriptions of the analyzed samples.

The three necropolises were first found and investigated in the mid-twentieth century. The first necropolis, the Monastery of Mostich, is in the outer southeastern area of Veliki Preslav, Shumen region. Its monastic identification is based on the burial inscription for the *icirgu-boilas* Mostich, a former military and administrative officer who later became a monk. He was reburied in a tomb in the north wall of the church. Three other tombs with buried and reburied monks were found in the south wall of the church. These tombs show evidence of burial practices typical for medieval Bulgarian monasteries. Another bipartite brick tomb, discovered in the western porch of the church, was affiliated to the noble monastic founder (George, the Bulgarian *synkellos*) and his closest relatives (Popkonstantinov and Kostova 2010, 2011, 2012, 2013).

Nojarevo, the second necropolis, is an early medieval necropolis characterized as pagan and biritual. Most graves are inhumation graves with specific corpse positioning and often with artificially deformed skulls and bones (Jordanov and Timeva 2010; Rashev 2008; Rashev et al. 1986, 1987, 1988, 1989).

The third necropolis is near the village of Tuhovishte in the southwestern Rhodope Mountains, Chech region. The stone graves are mostly inhumation, though some are cremation (Serafimova 1981).

The traditional methodology consisting in three fundamental steps was followed: i) PCR amplification of several short and overlapping target fragments to recover larger HVS I regions; ii) Production and sequencing of several clones for each amplified fragment; and iii) Alignment and comparison of sequences from different clones and different overlapping fragments to reconstruct the final consensus sequence of the entire region of interest (Rizzi et al. 2012).

All methods for preparing, extraction, and analyses of ancient mtDNA followed strict protocols (Hofreiter, Jaenicke et al. 2001; Paabo et al. 2004). The teeth were cleaned and powdered using a rotary tool, and mtDNA was extracted using a silica-based protocol (Caramelli et al. 2008; Hoss and Paabo 1993).

We analyzed sequences from hypervariable segment I (HVS-I) because most mtDNA variations belong to this region; it is also the region most commonly used for tracing human origins. Following the standard procedures (Caramelli et al. 2008; Pilli et al. 2013), we used AmpliTaq Gold<sup>®</sup> *Applied Biosystems*<sup>®</sup> to perform several steps of amplification and quantification of overlapping fragments covering 360 bp from HVS-I. The HVS-I was retrieved in three overlapping fragments (L15995-H16132, L16107-H16261 and L16247-H16402). For each step, the quality and quantity of fragments were checked by agarose gel electrophoresis. Amplified fragments were cloned using specific competent cells (*Escherichia coli*) and the TOPO TA Cloning<sup>®</sup> Kit, *Life technologies*<sup>™</sup>. Recombinant colonies were screened by PCR and purified by Microcon<sup>®</sup>, *EMD Millipore*<sup>®</sup> PCR purification. The products were Sanger sequenced using the BigDye<sup>®</sup> Terminator Kit, *Applied Biosystems*<sup>®</sup>.



Variation between samples was evaluated using *t* tests, and sample sequences from the separate clones of different amplicons were aligned and compared. Nucleotide changes occurring at particular positions in only one or two clones were considered amplification- or cloning-procedure errors. However, substitutions observed in a majority of clones were considered real mutations and were reported in the final consensus sequences. These ancient mtDNA variations were determined by aligning mtDNA sequences to the revised Cambridge reference sequence (rCRS). HVSI haplotypes were classified into possible haplogroups and sub-haplogroups using HaploGrep (Andrews et al. 1999; Hofreiter, Serre et al. 2001; Van Oven and Kayser 2009; Kloss-Brandstaetter et al. 2011).

The obtained haplogroup frequencies were compared with those in modern Eurasian populations, including the populations of Volga-Ural region by Principal Component Analysis (PCA) performed using Excel implemented with XIStat.

## **Results and Discussion**

From the analysis of 228 clone sequences we have obtained the mtDNA HVSI in 13 individuals, showing 12 independent haplotypes. They were further classified into 10 mtDNA haplogroups: H, H1, H5, H13, HV1, J, J1, T, T2 and U3 (Table 2; Achilli et al. 2007; Karachanak et al. 2012; Richards et al. 2002; Soares et al. 2010; Torroni et al. 2001).

We compared haplogroups in our ancient samples to those in modern Bulgarian samples previously analyzed (Karachanak et al., 2012).

The main haplogroup H, prevalent in European populations has 41.9 % frequency in modern Bulgarians (Karachanak, S. et al., 2012) and it is observed in seven out of thirteen proto-Bulgarian samples.

The rest of the ancient mtDNAs belong to one of the following Western Eurasian haplogroups HV1, J, J1, T, T2 and U3. They are found in modern Bulgarians with frequencies of: 0.2%, 7.9%, 1.3%, 10.6%, 6.3% and 1.9%, respectively. We found no evidence of East Asian (F, B, P, A, S, O, Y or M derivative) and African (L) haplogroups. Thus, our results do not support theories of Mongolo-Altaiic and Hun-Tataric origins of proto-Bulgarians.

The PCA analysis of modern Eurasian populations, including Volga-Ural populations and Proto-Bulgarians is based on mtDNA haplogroup frequencies given in Supplementary Table. The PCA plot (Fig. 2) shows that from mtDNA perspective the Proto-Bulgarians are positioned among South-Eastern and Southern European populations including modern Bulgarians. Proto-Bulgarians are genetically distant from Northern and Western Europeans and populations from the Near East and Caucasus. On the greatest distance from Proto-Bulgarians are Volga-Ural and Arabic populations.

Our results therefore suggest that proto-Bulgarians are genetically similar to modern Bulgarians and to certain South-Eastern European as well as Italian populations.

The future analyses of samples from human remains found on the territory of Bulgaria and dating to different periods (since III millennium BC) will further clarify the genetic make-up of past populations inhabiting modern Bulgarian lands.

## **Conclusion**

The range of the molecular anthropological research has increased in recent years due to the extensive research of the origins of modern and past populations. The results create a map of possible prehistoric human migration routes at different time scales and provide a detailed reconstruction of prehistoric and historic events all over the world. Thus, ancient and modern

data create a picture of our history since the appearance of modern humans 200,000 years ago in East Africa.

This work on ancient Bulgarian samples adds to the genetic picture of the past by presenting the first data on ancient mtDNA samples from individuals who inhabited the current Bulgarian territories from VIII-X century AD. Our results show that the haplogroups found in ancient samples are predominantly Western Eurasians. This finding supports the concept for the Western Eurasian matrilineal origins of the Proto-Bulgarians and is controversial to the Mongolo-Altaic and Hun-Tataric theories. The comparison of Proto-Bulgarians and modern Eurasian populations, including those from the Volga-Ural region shows that despite the time gap of more than eleven centuries, there is a genetic similarity between proto- and modern Bulgarians (Karachanak et al. 2012).

Phylogenetic analysis of additional human remains will help to further clarify the gradual changes in the matrilineal composition of past populations inhabiting modern Bulgarian lands. This data will contribute to a deeper understanding of the Bulgarian genetic past.

## **Acknowledgments**

This study was supported by the National Science Fund of Bulgaria, project “Characterization of the anthropo-genetic identity of Bulgarians,” contract number DOO 2-110/22.05.2009.

*Received 22 December 2014; revision accepted for publication 11 April 2015.*

## **Literature Cited**

- Achilli, A., A. Olivieri, M. Pala et al. 2007. Mitochondrial DNA variation of modern Tuscans supports the near eastern origin of Etruscans. *Am. J. Hum. Genet.* 80:759–768.
- Adachia, N., K. Umetsu, W. Takigawa et al. 2004. Phylogenetic analysis of the human ancient mitochondrial DNA. *J. Archaeol. Sci.* 31:1,339–1,348.
- Andrews, R.M., I. Kubacka, P. F. Chinnery et al. 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat Genet* 23:147.
- Bazin, L. 1974. *Les calendriers turcs anciens et médiévaux*. Thèse, Paris III, 1974: Université de Lille.
- Beshevliev, V. 1993. First Bulgarian inscriptions. 2nd ed. Sofia: *Bulgarian Academy of Sciences*.  
(Бешевлиев, В. 1993. Първите Български надписи. 2-ро издание. София. Българска Академия на Науките.)
- Caramelli, D., L. Milani, S. Vai et al. 2008. A 28,000 years old Cro-Magnon mtDNA sequence differs from all potentially contaminating modern sequences. *PLoS One* 3:e2700.
- Daskalov, R. 2011. The amazing world of the proto-Bulgarians. Sofia: *Gutenberg Publishing House*.  
(Даскалов, Р. 2011. Невероятният свят на Прабългарите. София: *Издателска къща Гутенберг*.)
- Dimitrov, B. 2005. 12 myths in Bulgarian history. Sofia: *KOM Foundation*.  
(Димитров, Б. 2005. 12 мита в Българската история. София: Фондация КОМ.)
- Dujchev, I., ed. 1963. The Medieval translation of the Chronicle of Manassius. Sofia: *Bulgarian Academy of Sciences*.  
(Дуйчев, И., едит. 1963. Средновековен превод на Манасиевите хроники. София: *Българска Академия на Науките*.)
- Dobrev, P. 1991. The proto-Bulgarians: Origin, language, culture. Sofia: *Proxima*.

(Добрев, П. 1991. Прабългарите: Произход, език, култура. София: Издателство *Проксима*.)

Dobrev, P. 1998. Crucified history. Part I: Delusions of the 19th century. Sofia: *ИКК "Slavika-  
RM."*

(Добрев, П. 1998. История разпъната на кръст. Част I: Заблудите на 19-ти век. София: *ИКК  
„Славика- РМ.“*)

Dobrev, P. 2005. The golden core of the Bulgarian antiquity. Sofia: *Tangra TanNakRa IK.*

(Добрев, П. 2005. Златното ядро на Българската античност. София: *Тангра. ТанНакРа ИК.*)

Fol, A., G. Bakalov, P. Dobrev et al. eds. 2000. The Bulgarians. Sofia: *Tangra TanNakRa.*

(Фол, А., Г. Бакалов, П. Добрев и съавтори. 2000. Българите. София: *ТанНакРа.*)

Forster, P. 2004. Ice Ages and the mitochondrial DNA chronology of human dispersals: A  
review, with discussion. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 359:255–264.

Haefs, H. 2009. *Das goldene Reich der Pamir-Bulgaren an Donau und Wardare*: Books on  
Demand.

Hofreiter, M., V. Jaenicke, D. Serre, et al. 2001. DNA sequences from multiple amplifications  
reveal artifacts induced by cytosine deamination in ancient DNA. *Nucleic Acids Res.*  
29:4,793–4,799.

Hofreiter, M., D. Serre, H. N. Poinar et al. 2001. Ancient DNA. *Nature Reviews. Genetics* 2:353–  
359.

Hoss, H., and S. Paabo. 1993. DNA extraction from Pleistocene bones by a silica based  
purification method. *Nucleic Acids Res.* 21:3,913–3,914.

Jireček, K. 1878. *History of the Bulgarians*. Odessa: V. Aprilov.

Jireček, K. 1876. *Dějiny národa bulharského*. Prague: Nákladem B. Tempského.

Jireček, C. 1876. *Geschichte der Bulgaren*. Prague: F. Tempsky.

- Jordanov J., and N. Timeva. 2010. Preliminary data of anthropological approach of early medieval pagan necropolis near Nojarevo village, Silistra region. Scientific reports of III national conferences, From regional to national: “Numismatics, Sfragistics, Epigraphy and Museology,” Historical Museum Polski Trambesh, *Abagard*: 33–40.
- (Йорданов Й., и Н. Тимева. 2010. Първоначални данни на антропологично изследване на ранносредновековни езически некрополи близо до село Ножрево, Силистренска област. Научноизследователски доклад на 3-ти национална конференция, От регионалното към националното: „ Нумизматика, Сфрагистика, Епиграфика и Музейно дело,“ Исторически музей Полски Трамбеш, *Абагард*: 33–40.)
- Karachanak, S., V. Carossa, D. Nesheva et al. 2012. Bulgarians vs the other European populations: A mitochondrial DNA perspective. *Int. J. Legal Med.* 126:497–503.
- Kloss-Brandstaetter, A., D.Pacher, S.Schoenherr et al. 2011. HaploGrep: a fast and reliable algorithm for automatic classification of mitochondrial DNA haplogroups. *Hum. Mut.* 32:25-32
- Manchen-Helfen, O. 1973. *The world of the Huns: Studies in their history and culture*. Berkeley: University of California Press.
- Menges, K. H. 1968. *The Turkic languages and peoples: An introduction to Turkic studies*. Wiesbaden: Otto Harrassowitz.
- Paabo, S., H. Poinar, D. Serre et al. 2004. Genetic analyses from ancient DNA. *Annu. Rev. Genet.* 38:645–679.
- Petrov, P., and V. Gjuzelev, eds. 1979. *Chrestomathy of Bulgarian history*, vol. I. Sofia: Bulgarian Writer Editing House, 88–125.
- (Петров, П., и В. Гюзелев, едс. 1979. *Христоматия на Българската история*, Том I. София: Българска Издателска Къща, 88-125.)

- Pilli, E., A. Modi, C. Serpico et al. 2013. Monitoring DNA contamination in handled vs. directly excavated ancient human skeletal remains. *PLoS One* 8:e52524.
- Pritsak, O. 1955. *Die Bulgarische Fürstenliste und die Sprache der Protobulgaren*. Wiesbaden: Ural-Altäische Bibliothek, I.
- Popkonstantinov, K., and R. Kostova. 2010. Monastery of chergoboil Mostich, m. Selishte, V. Preslav. *Archaeological Discoveries and Excavations* 474–477.
- (Попконстантинов, К., и Р. Костова. 2010. Манастира на чергобоил Мостич, м. Селище, В. Преслав. *Археологични открития и разкопки* 474–477.)
- Popkonstantinov, K., and R. Kostova. 2011. Monastery of chergoboil Mostich, m. Selishte, V. Preslav *Archaeological Discoveries and Excavations* 419–422.
- (Попконстантинов, К., и Р. Костова. 2011. Манастира на чергобоил Мостич, м. Селище, В. Преслав. *Археологични открития и разкопки* 419–422.)
- Popkonstantinov, K., and R. Kostova. 2012. Monastery of chergoboil Mostich, m. Selishte, V. Preslav. *Archaeological discoveries and excavations* 384–387.
- (Попконстантинов, К., и Р. Костова. 2012. Манастира на чергобоил Мостич, м. Селище, В. Преслав. *Археологични открития и разкопки* 384–387.)
- Popkonstantinov, K., and R. Kostova. 2013. Manastery of Georg, synkellos of Bulgarians in Preslav: History of one Bulgarian aristocratic family of X century. *Preslav, Veliki Preslav municipality, NIAM AT BAS - Shoumen* 7:44–63.
- (Попконстантинов, К., и Р. Костова. 2013. Манастира на Георги, „Синкел“ на българите в Преслав: История на едно българско аристократично семейство от 10-ти Век. *Преслав, Община Велики Преслав, НИИМ АТ БАН – Шумен* 7:44–63.)
- Rashev R., 1993. About the origin of proto-Bulgarians. In *Studia Protobulgarica et Mediaevalia Europensia* (In honor of Professor Vesselin Beshevliev). *Veliko Tarnovo*.

(Рашев, Р., 1993. Относно произхода на Прабългарите. В „*Изучаване на Прабългарите в Средновековна Европа*“ ( В чест на Професор Веселин Бешевлиев). *Велико Търново.*)

Rashev, R. 2008. *Bulgarian pagan culture VII–IX century. Sofia.*

(Рашев, Р., 2008. *Българската езическа култура 7-ми – 9-ти Век.* Издателство София.)

Rashev R., S. Stanilov, and G. Atanasov. 1986. Proto-Bulgarian pagan necropolis and village near Nojarevo village, Silistra region. *Archaeological Discoveries and Excavations in 1985, Veliko Turnovo.*

(Рашев, Р., С. Станилов, и Г. Атанасов. 1986. Прабългарски езически некропол и селище в близост до село Ножарево, Силистренски регион. *Археологични открития и разкопки през 1985, Велико Търново.*)

Rashev R., S. Stanilov, and G. Atanasov. 1987. Early medieval necropolis near Nojarevo village, Silistra region. *Archaeological Discoveries and Excavations in 1986, Razgrad.*

(Рашев, Р., С. Станилов, и Г. Атанасов. 1987. Ранносредновековен некропол близо до село Ножарево, Силистренски регион. *Археологични открития и разкопки през 1986, Разград.*)

Rashev R., S. Stanilov, and G. Atanasov. 1988. Early medieval necropolis near Nojarevo village, Silistra region. *Archaeological Discoveries and Excavations in 1987, Blagoevgrad.*

(Рашев, Р., С. Станилов, и Г. Атанасов. 1988. Ранносредновековен некропол близо до село Ножарево, Силистренски регион. *Археологични открития и разкопки през 1987, Благоевград.*)

Rashev R., S. Stanilov, and G. Atanasov. 1989. Proto-Bulgarian pagan necropolis near Nojarevo village, Silistra region. *Archaeological Discoveries and Excavations in 1988, Kurdjali.*



- (Рашев, Р., С. Станилов, и Г. Атанасов. 1989. Прабългарски езически некропол в близост до село Ножарево, Силистренски регион. *Археологични открития и разкопки през 1988, Кърджали.*)
- Richards, M., V. Macaulay, A. Torroni et al. 2002. In search of geographical patterns in European mitochondrial DNA. *Am. J. Hum. Genet.* 71:1,168–1,174.
- Rizzi, E., M. Lari, E. Gigli et al. 2012. Ancient DNA studies: new perspectives on old samples. *Genet. Sel. Evol.* 44:21.
- Serafimova, D. 1981. Early medieval necropolis near Tuhovishta village, Blagoevgrad. DI Septemvri (Brochure from regional historical museum—Blagoevgrad).
- (Серафимова, Д. 1981. Ранносредновековен некропол близо до село Туховище, Благоевград. ДИ Септември (Брошура от регионалния исторически музей-Благоевград).)
- Slatarski, V. 1918. Geschichte der Bulgaren I: Von der Gründung des bulgarischen Reiches bis zur Türkenzeit (679–1396). *Bulgarische Bibliothek* 5, Leipzig.
- Soares, P., A. Achilli, O. Semino et al. 2010. The archaeogenetics of Europe. *Curr. Biol.* 20:R174–183.
- Stamatov, A. 1997. *Tempora incognita of the early Bulgarian history*. Sofia: UMG “St. Ivan Rilski.”
- (Стаматов, А. 1997. *Неизяснени периоди в ранната Българска история*. София: УМГ „Св. Иван Рилски.“)
- Torroni, A., H.-J. Bandelt, V. Macaulay et al. 2001. A signal, from human mtDNA, of postglacial recolonization in Europe. *Am. J. Hum. Genet.* 69:844–852.
- Van Oven, M., and M. Kayser. 2009. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum. Mutat.* 30:E386–394.

- Yao, Y. G., and Y. P. Zhang. 2000. Mitochondrial DNA and human evolution. *Zoological Research (in Chinese)* 21:392–406.
- Yonggang, Y., and Z. Yaping. 2003. Pitfalls in the analysis of ancient human mtDNA. *Chinese Science Bulletin* 48:826–830.
- Zlatarski, V. 1914. History of the Bulgarians from their appearance in Europe by the founding of the Bulgarian kingdom on the Balkan Peninsula. *Annual of Sofia University Historical-Philological Faculty* 10–11:1–112.
- (Златарски, В. 1914. История на българите от появата им в Европа до основаването на българското царство на Балканския полуостров. *Годишник на Софийския Университет, Исторически-филологическия факултет* 10–11:1–112.)
- Zlatarski, V. 1918. *History of the Bulgarian state in the Middle Ages*, vol. 1, *First Bulgarian Kingdom: Age of Hun-Bulgarian dominance (679–852)*, 3rd ed. Sofia.
- (Златарски, В. 1918. *История на Българската държава през Средните векове*, Том 1, *Първото Българско царство: Периода на Хуно-Българското доминиране*. (679–852), 3-то издание. София.)
- Zlatarski, V. 1970. *History of the Bulgarian state in the Middle Ages*, vol. 1, *First Bulgarian Kingdom: Age of Hun-Bulgarian dominance*, Sofia.
- (Златарски, В. 1970. *История на Българската държава през Средните векове*, Том 1, *Първото Българско царство: Периода на Хуно-Българското доминиране*, София.)

**Table 1. Description of Samples from Three Necropolises in Bulgaria**

<b>Sample</b>	<b>Necropolis</b>	<b>Grave</b>	<b>Location/Description</b>	<b>Dating</b>
NJ 50	Nojarevo	No. 50		
NJ 54	Nojarevo	No. 54	Northeast Bulgaria	
NJ 84	Nojarevo	No. 84	Nojarevo Village/ Proto-Bulgarian	VIII-IX Century
NJ 77	Nojarevo	No. 77	Separate graves	
NJ 125	Nojarevo	No. 125		
TUH 448	Tuhovishte	No. 448		
TUH 449	Tuhovishte	No. 449	Southwest Bulgaria	
TUH 1649	Tuhovishte	No. 1649	Tuhovishte Village/ Proto-Bulgarian	IX-X Century
TUH 1652	Tuhovishte	No. 1652	Separate graves	
TUH 1665	Tuhovishte	No. 1665		
MM 1.2	Monastery of Mostich	No. 5	Northeast Bulgaria	
MM 1.3	Monastery of Mostich	No. 25	Veliki Preslav/ Proto-Bulgarian	X Century
MM 1.4	Monastery of Mostich	No. 5	Separate graves	

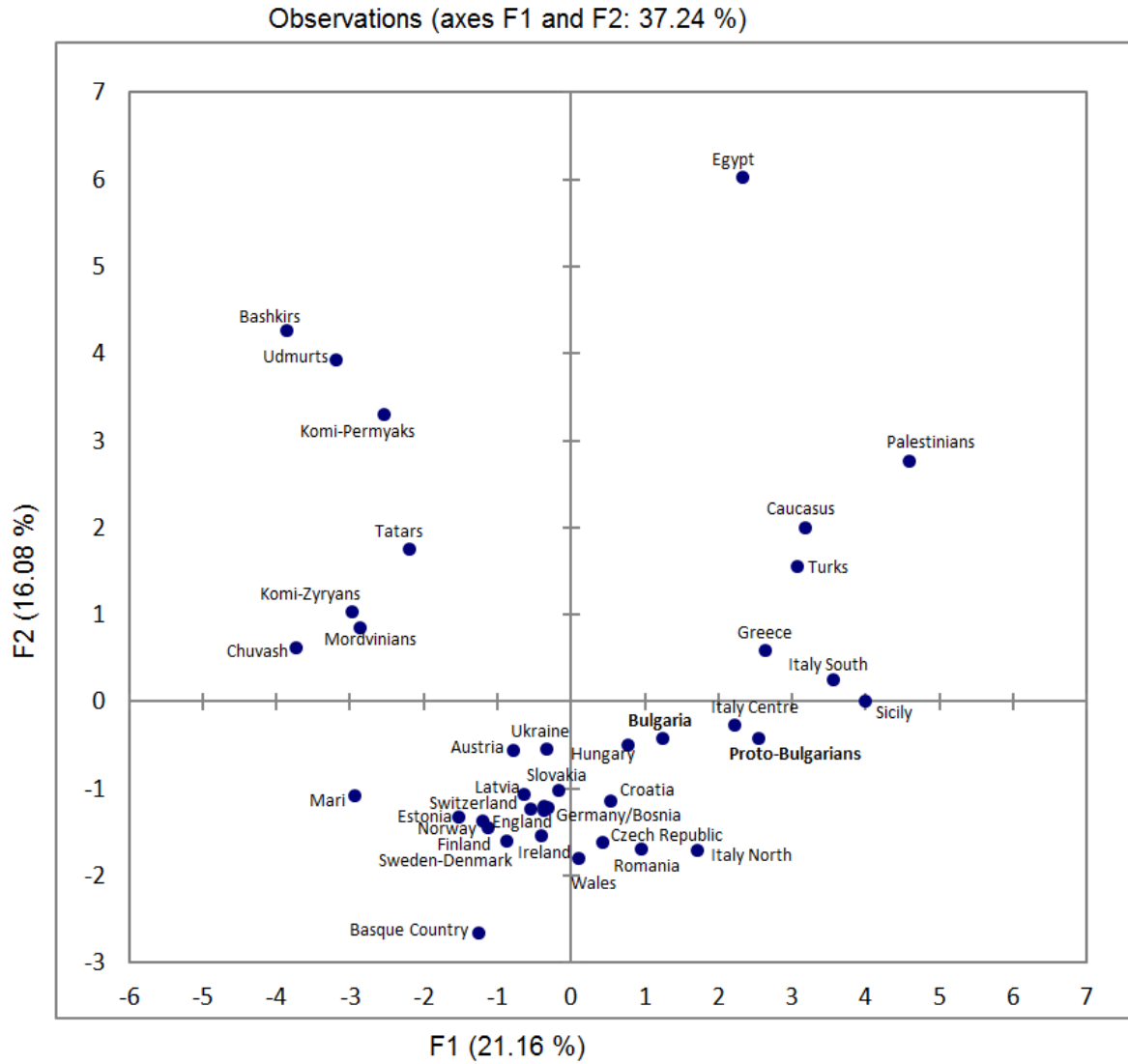
**Table 2. Haplotypes and Haplogroups in Proto-Bulgarians**

Sample	Clones	HVS-I haplotypes	Sequence range	Hg	Sub-Hg	HaploGroup quality score
MM 1.2	19	092 142 266 278	16024-16383	H1	H1t1a1	60
MM 1.3	15	343	16225-16383	U3	U3	100
MM 1.4	19	069 126	16024-16281	J	J	100
NJ 50	18	111 173 183 278	16024-16383	H1	H1r1	61
NJ 54	18	069 126 145 172 222 261	16024-16383	J1	J1b1a1	100
NJ 77	10	055 067	16024-16156; 16225- 16383	HV1	HV1	100
NJ 84	22	126 274 294 296	16024-16383	T2	T2	91
NJ 125	14	CRS	16024-16281	H	H2a2a1	0
TUH 448	11	148 256	16024-16281	H13	H13a2c 1	100
TUH 449	22	304	16024-16383	H5	H5	100
TUH 1649	19	CRS	16024-16383	H	H2a2a1	0
TUH 1652	18	126 294	16024-16383	T	T	100
TUH 1665	23	219 325	16024-16383	H1	H1a2	69

MM = Monastery of Mostich necropolis; NJ = Nojarevo necropolis; TUH = Tuhovishte necropolis; Hg = haplogroup; CRS = Cambridge reference sequence



**Figure 1.** Physical map of Bulgaria showing the location of the three necropolises from which the human remains were collected (Nojarevo dated to VIII-IX century, Tuhovishte-IX-X and Monastery of Mostich – X century).



**Figure 2.** Plot of the Principal Component Analysis based on mtDNA haplogroup frequencies in Eurasian populations.

**Supplementary Table.** Populations included in the PCA, number of samples analyzed (N) and number of individuals belonging to mtDNA haplogroups.

Popu l.	Refer ences	N	H*	H5	HV 0	HV	R0a	JT	U1	U2e	U3	U4	U5a	U5b	U6	U7	U8	U*	K	N1	N2	X	M	L	Othe rs
Austr ia	Karach anak et al., 2012	99	43	3	1	1	1	20*	0	1	1	4	8	0	0	0	2	1	7	2	1	1	2	0	0
Basq ue Coun try	Karach anak et al., 2012	156	87	6	17	0	0	13*	0	0	0	0	2	17	0	1	1	0	6	0	0	2	0	1	3
Bosni a	Karach anak et al., 2012	144	61	8	9	0	2	17*	2	0	1	8	10	7	0	0	0	0	6	5	2	2	2	1	1
Bulg aria	Karach anak et al., 2012	996	380	33	35	39	6	182*	13	10	21	39	45	25	0	7	4	3	59	27	25	20	11	4	8
Cauc asus	Karach anak et al., 2012	2650	573	61	24	104	7	439*	108	60	147	104	153	27	1	25	13	1	159	56	93	145	163	2	185
Croat ia	Karach anak et al., 2012	96	36	7	5	3	0	9*	1	4	2	2	8	2	0	1	0	0	6	3	4	0	2	0	1
Czec h Repu	Karach anak et al.,	83	28	6	5	3	0	18*	0	1	1	1	7	3	0	0	0	0	3	3	1	3	0	0	0

blic	2012																								
Egypt	Karachanak et al., 2012	413	15	2	13	14	7	55*	2	1	14	5	1	15	3	2	0	0	23	27	4	7	41	94	68
England	Karachanak et al., 2012	335	148	13	11	1	0	75*	0	3	2	7	13	15	0	1	1	0	21	10	3	3	0	2	6
Estonia	Karachanak et al., 2012	558	235	17	18	6	0	98*	1	7	5	32	56	24	0	0	9	1	15	13	15	5	1	0	0
Finland	Karachanak et al., 2012	312	113	8	19	0	0	37*	1	2	0	5	18	44	0	1	0	0	19	17	16	6	0	1	5
Germany	Karachanak et al., 2012	905	368	43	37	3	0	177*	4	2	16	26	46	35	0	2	2	3	63	24	21	10	2	1	20
Greece	Karachanak et al., 2012	155	54	6	3	5	3	31*	3	1	3	4	7	4	0	2	0	1	7	7	2	7	4	0	1
Hungary	Karachanak et al., 2012	533	136	21	25	4	7	75*	4	4	1	9	15	17	1	1	11	2	113	28	28	7	11	2	11
Ireland	Karachanak et al., 2012	300	126	5	17	4	1	54*	0	4	3	4	11	15	0	0	0	0	37	9	7	2	1	0	0



Italy Centr e	Karach anak et al., 2012	12 73	427	52	61	45	14	251 *	11	10	34	21	56	34	4	14	10	1	84	35	25	39	25	18	2
Italy North	Karach anak et al., 2012	34 6	131	33	18	8	1	53*	3	5	4	10	7	4	0	0	1	0	32	12	6	17	1	0	0
Italy South	Karach anak et al., 2012	53 9	209	25	19	18	5	93*	10	4	17	13	11	7	7	6	2	0	39	21	9	14	7	1	2
Latvi a	Karach anak et al., 2012	29 9	113	20	9	7	0	47*	0	9	5	28	21	6	0	0	0	0	7	13	12	1	1	0	0
Norw ay	Karach anak et al., 2012	55 6	250	17	21	1	0	106 *	1	0	8	17	35	31	0	0	2	0	31	13	9	2	6	0	6
Pales tinian s	Karach anak et al., 2012	11 7	28	5	0	2	3	26*	1	1	1	2	1	0	1	3	0	1	9	3	3	4	4	16	3
Rom ania	Karach anak et al., 2012	94	26	7	6	1	2	21*	0	0	3	4	7	5	0	0	0	0	3	0	6	2	0	0	1
Sicily	Karach anak et al., 2012	10 5	51	6	3	4	2	13*	2	1	1	3	1	1	1	2	0	0	6	3	0	3	0	2	0
Slova	Karach	12	49	9	3	2	0	30*	0	2	3	2	9	2	0	0	0	0	5	6	3	0	1	0	3

kia	anak et al., 2012	9																							
Sweden-Denmark	Karachanak et al., 2012	75	31	4	3	0	0	16*	0	1	0	3	5	0	0	0	0	0	8	1	1	0	0	0	2
Switzerland	Karachanak et al., 2012	228	93	11	11	1	0	54*	0	2	2	8	11	5	1	0	1	0	12	3	4	1	0	1	7
Turks	Karachanak et al., 2012	340	99	10	2	17	0	61*	11	4	19	5	4	6	0	2	4	2	19	13	10	15	15	7	15
Wales	Karachanak et al., 2012	92	43	7	3	2	0	18*	0	0	0	0	3	3	0	0	0	0	7	3	0	1	0	0	2
Bashkirs	Bermisheva et al. 2002	221	25	2	7	1	0	19	0	0	0	28	15	15	0	0	1	0	3	11	1	0	61	0	33
Tatars	Bermisheva et al. 2002	228	68	2	9	1	0	38	2	0	5	16	20	4	0	0	0	5	13	7	4	0	20	0	17
Chuvash	Bermisheva et al. 2002	55	15	0	4	0	0	5	0	0	1	9	8	0	0	0	1	1	4	2	1	0	4	0	0
Mordvinia	Bermisheva et al.	102	42	1	5	1	0	16	0	1	0	2	7	9	0	0	0	2	0	6	0	0	3	0	7

ns	al. 2002																									
Komi - Perm yaks	Bermisheva et al. 2002	74	22	2	0	0	0	13	0	0	0	7	1	3	0	0	0	1	1	9	0	0	12	0	6	
Komi - Zyryans	Bermisheva et al. 2002	62	21	0	0	0	0	14	0	0	0	15	2	4	0	0	1	0	1	0	1	0	2	0	5	
Mari	Bermisheva et al. 2002	136	54	1	15	2	0	17	0	0	0	14	17	2	0	0	0	0	3	1	0	0	8	0	2	
Udmurts	Bermisheva et al. 2002	101	21	1	0	0	0	23	0	5	0	4	8	1	0	0	0	2	0	0	0	0	20	0	11	
Ukraine	unpublished	110	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub	unpub.	
Protobulgarians	present study	13	6	1	0	1	0	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	

Karachanak, S., V. Carossa, D. Nesheva et al. 2012. Bulgarians vs the other European populations: A mitochondrial DNA perspective.

*Int. J. Legal Med.* 126:497–503.

Bermisheva, M. A., K. Tambets, R. Villems et al. 2002. Diversity of Mitochondrial DNA Haplogroups in Ethnic Populations of the Volga–Ural Region. *Mol. Biol.* 36:802–812.

\*Haplogroup JT includes haplogroup J, T1 and T2