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REVIEW

Global trends in DNA research on aged human skeletal remains: a bibliometric analysis (1989–2024)

Jiao Luo[1] · Zhiqi Hua[1] · Ji Chen[1] · Qi Yang[1] · Qi Wei[1] · Sitong Liu[1] · Yongjie Cao[1] · Anqi Chen[1] · Chengtao Li[1] · Ranran Zhang[1] · Suhua Zhang[1]

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Abstract

The DNA analysis of aged human skeletal remains is pivotal to forensic genetics, forensic anthropology, and archaeology. Using VOSviewer and Biblioshiny, we analyzed 982 publications from the Web of Science (WoS) Core Collection to evaluate publication trends, key contributors, influential journals and articles, and the thematic evolution of the field through co-authorship networks, keyword co-occurrence, and temporal overlay mapping. The results indicate the publication in this field began in 1989 and peaked at 72 articles in 2021. The most prolific countries were the United States, Germany, the United Kingdom, Italy, and Spain. Leading institutions included the Max Planck Institute for Evolutionary Anthropology, the University of Ljubljana, and the University of Copenhagen. High-impact contributors included Pääbo, Svante; Willerslev, Eske; Krause, Johannes; and Pajnič, Irena Zupanič. The most active journals were *Forensic Science International*, *Journal of Forensic Sciences*, *Forensic Science International: Genetics*, *International Journal of Legal Medicine*, and *American Journal of Physical Anthropology*. The most highly cited article was "Hofreiter, 2001, Nat Rev Genet". Keywords co-occurrence highlighted "ancient DNA (aDNA)", "human skeletal remains", "mitochondrial DNA (mtDNA)", "forensic science", and "human identification" as dominant themes. Recent trends underscore the integration of aDNA into forensics workflows, increasing reliance on mtDNA and single nucleotide polymorphism (SNP) profiling, and massively parallel sequencing (MPS) applications for degraded DNA. Collectively, the findings highlight the transformative impact of technological innovation and international collaboration on the field's evolution. Continued global cooperation is essential to drive innovation, optimize resources, and accelerate forensic genetics research.

Keywords Forensic science · Forensic genetics · Human skeletal remains · DNA · Bibliometrics

Introduction

Human skeletal remains constitute a critical source of endogenous DNA in forensic genetics, anthropology, archaeology, and related disciplines [1]. In contexts such as battlefield remains recovery, missing person identification (MPI), historical case resolution, disaster victim identification (DVI), humanitarian work, and cases involving migration or human trafficking, skeletal elements and teeth are often the sole viable sources of endogenous DNA. Over the past two decades, global initiatives to recover and identify war casualties [2], disaster victims [3], and historical figures [4] have intensified scientific interest in the genetic analysis of skeletal remains. The integration of massively parallel sequencing (MPS) has revolutionized the field, significantly enhancing the sensitivity, resolution, and throughput of DNA profiling from degraded samples.

The pioneering study by Hagelberg E et al. published in *Nature* in 1989, successfully amplified skeletal DNA using polymerase chain reaction (PCR) technology [5]. In 1991, the same team further applied skeletal DNA analysis in the fields of human identification and forensic investigation [6]. Since then, DNA analysis of aged skeletal remains has become indispensable in modern forensic casework

Jiao Luo and Zhiqi Hua contributed equally to this work.

✉ Ranran Zhang
zhangranran@fudan.edu.cn

✉ Suhua Zhang
zhang_suhua@fudan.edu.cn

[1] Institute of Forensic Science, Fudan University, No. 131 Dongan Road, Xuhui District, Shanghai 200032, PR China

(Fig. 1). Its applications are diverse: resolving unidentified human remains (UHR) cases from conflicts and disasters, verifying historical individuals to reconstruct biographical narratives, supporting criminal investigations by providing genetic evidence, supporting humanitarian works, tracing population origins, identifying victims of human trafficking, and strengthening transnational judicial investigations [7]. Beyond forensic identification, this approach also contributes to population genetics, studies of human migration, postmortem interval (PMI) estimation, and the refinement of forensic methodologies. These milestones laid the groundwork for contemporary techniques, such as MPS and advanced bioinformatics, which now play a central role in the analysis of degraded DNA.

Technology advancements and increasing demands for postmortem identification have expanded the scope of skeletal DNA applications in the 21 st century. Key forensic and anthropological uses now include (1) Individual identification, utilizing autosomal STRs (aSTRs), Y-STRs, X-STRs, miniSTRs, insertion/deletion polymorphisms (InDels), and identity-informative SNPs (iiSNPs) [8]; (2) Forensic DNA phenotyping, involving the prediction of externally visible characteristics (EVCs) (e.g., pigmentation, facial features) through phenotype-informative SNPs (piSNPs) [9], and age estimation via age-associated CpG methylation profiling [10]; (3) Kinship analysis, to assist familial identification efforts [11]; (4) BGAI based on ancestry-informative markers (AIMs), Y-chromosome haplogroups, and mitochondrial haplotypes [9, 12]; (5) Forensic genetic genealogy (FGG), a rapidly emerging tool for investigative lead generation [13]; (6) Skeletal microbiome and virome study provide new tools for the investigation of human skeletal remains in forensic and archaeological contexts [14]; (7) Targeted identification databases, designed to catalog profiles for missing persons and unidentified remains [7]. These diverse applications underscore the multidisciplinary nature of the skeletal DNA research, which lies at the intersection of forensic genetics, anthropology, archaeology, and human population studies.

Many of the analytical strategies employed in skeletal DNA research originate from protocols developed in ancient DNA (aDNA) studies [15]. The standard workflow comprises four major steps (Supplementary Fig.S1): (1) Sample recovery and pretreatment. Research in archaeology, anthropology, and forensics establishes skeletons and teeth as more prevalent at scenes than hair. Their resilient mineral structure resists degradation and provides higher DNA yield, making them the preferred elements [16]. These samples are processed under strict

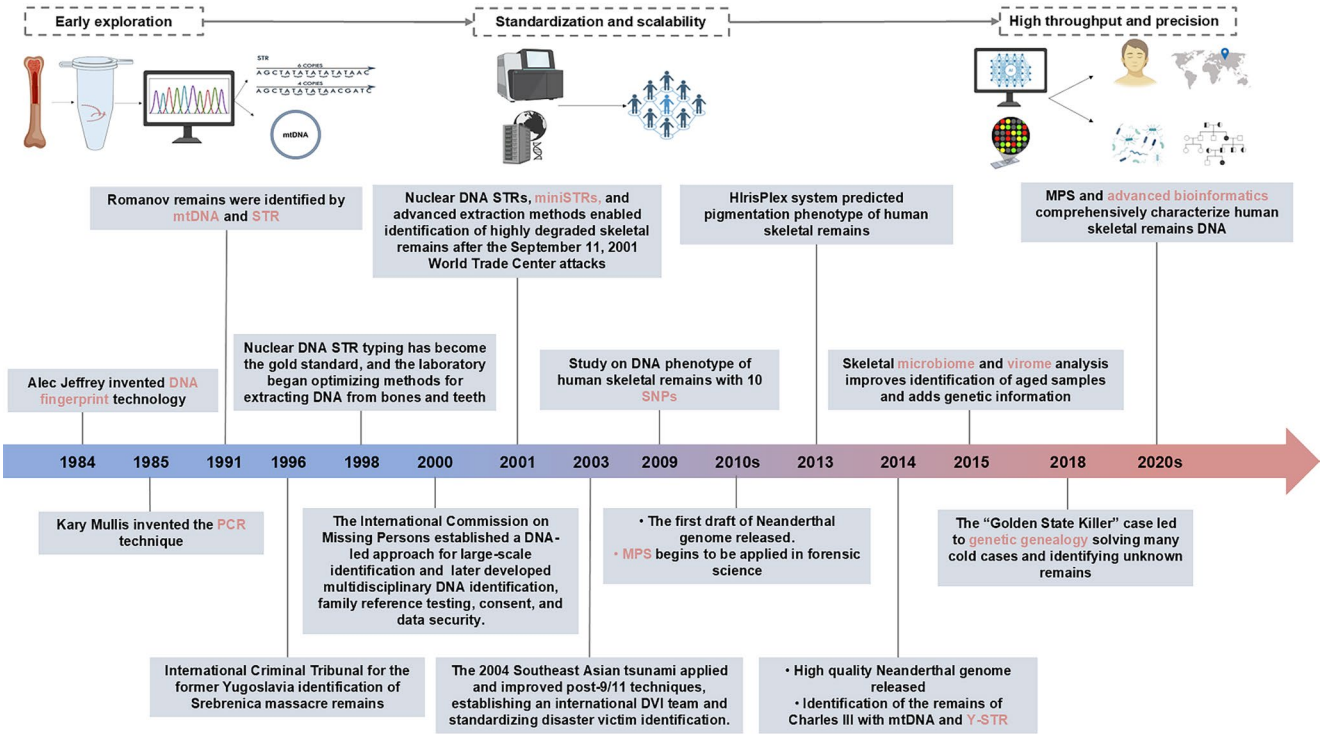

Fig. 1 Historical milestones in DNA analysis of aged human skeletal remains. The evolution of DNA analysis from aged human skeletal remains can be broadly divided into three phases: (1) Early exploration. Initial efforts focused on recovering degraded DNA using PCR, Sanger sequencing with fluorescent detection; (2) Standardization and scalability. The introduction of STR profiling and bioinformatics tools, commercial kits, and protocol standardization enabled broader forensic applications and result interpretation; (3) High throughput and precision. The implementation of MPS, SNP-based profiling, and advanced bioinformatics has significantly improved sensitivity, resolution, and data integration. Progress in genetic markers and analytical techniques has driven this evolution

anti-contamination conditions. Common decontamination procedures include treatment with bleach, ultraviolet (UV) irradiation, and mechanical removal of surface contaminants. Rigorous negative controls are employed throughout to monitor potential contamination [17]. (2) DNA extraction and quantification. Although no single protocol is universally accepted, widely used methods include magnetic bead-based extraction, organic extraction, and silica column-based protocols [18]. (3) Genetic markers typing and mitochondrial DNA (mtDNA) sequencing. In addition to traditional capillary electrophoresis (CE) and microarray platforms, MPS has enabled higher throughput and resolution analysis of degraded DNA samples [19]. (4) Data analysis and interpretation. Informative alleles and haplotypes are identified and analyzed using forensic databases and bioinformatics tools to support forensic DNA phenotyping (FDP), BGAI, individual identification, and kinship analysis.

In recent years, bibliometric analysis has emerged as a valuable method for mapping scientific landscapes by quantifying publication trends, thematic development, and collaborative networks [20]. Within forensic science, bibliometric approaches have provided strategic insights into the evolution and interdisciplinarity of various subfields. However, despite the growing relevance of aged human skeletal DNA analysis, systematic bibliometric evaluations of this research domain remain limited. To address this gap, the present study conducts a comprehensive bibliometric analysis of publications related to DNA analysis of aged human skeletal remains indexed in the Web of Science (WoS) Core Collection from 1989 to 2024. Using VOSviewer, Biblioshiny (RStudio), we investigate trends in publication and citation dynamics, country and organization contributions, key authors and journals, thematic evolution, and co-occurrence of author keywords. A three-field plot further explores the interconnection among journals, countries, and author keywords. To provide a comprehensive overview of research progress in this field, all included publications are compiled as an appendix (Supplementary Appendix 1) and categorized by topic, thereby highlighting the development and key focus of each research area.

This study aims to elucidate the structural development of this niche yet impactful research field, offering a consolidated reference for forensic scientists, anthropologists, and geneticists. It provides a roadmap for understanding the past, present, and future directions of skeletal DNA research within the broader context of forensic science and human identification. Furthermore, the findings of this study may reveal current international data-sharing and collaborative patterns, thereby advancing more equitable and sustainable forensic science.

Materials and methods

Database and search strategy

The WoS is a widely recognized academic database that indexes high-quality publications across various disciplines, rendering it well-suited for bibliometric analysis [21]. To identify all English-language studies on DNA analysis of aged human skeletal remains published up to 2024, we conducted three rounds of systematic literature searches. The initial search was performed between October and December 2024 and the last in August 2025. Publications from 2025 to 2026 were excluded due to incomplete indexing at the time of analysis.

The search strategy began by accessing the official WoS platform, selecting the Core Collection with the Science Citation Index Expanded (SCI-Expanded, 1900–present) and Social Sciences Citation Index (SSCI, 1990–present) editions. To maximize coverage of relevant studies, three distinct search strategies were employed (Fig. 2). Specifically, the queries included: (1)"All Fields" = "human skeletal remains DNA" OR "human bone remains DNA"; (2) Topic (TS) = ("human remains" OR "skeletal remains" AND DNA); and (3)"All Fields" = "teeth DNA" OR "tooth DNA" OR "dental DNA" AND "Subject Category" = "Legal Medicine". These strategies yielded 3,787, 834, and 457 records, respectively. Filters were then applied to restrict document types to "Article" and "Review Article", limit the language to English, and exclude publications from 2025 to 2026, resulting in a final dataset of 3,658 records. Titles and abstracts were screened for relevance to the study topic, and relevant records were exported. Following merging and deduplication using Python v3.13, 1,522 unique records remained. Subsequently, three independent investigators conducted a detailed evaluation of the titles and abstracts of all remaining records to confirm their relevance. When necessary, full-text articles were reviewed to determine eligibility. Ultimately, 982 articles met the inclusion criteria and were included in the analysis.

Data preparation

Author keywords were extracted using VOSviewer v.1.6.20.0 and subsequently standardized according to the following rules: (1) synonyms were merged, for example, both "ancient DNA" and "ancient-DNA" were unified as "aDNA"; (2) letter case was standardized, with abbreviations of forensic science terms consistently presented in uppercase (e.g., "DNA", "SNP"); (3) common forensic abbreviations were adopted in place of full terms; for instance, "short tandem repeat" and "single nucleotide polymorphism" were converted to "STR" and "SNP," respectively, while other

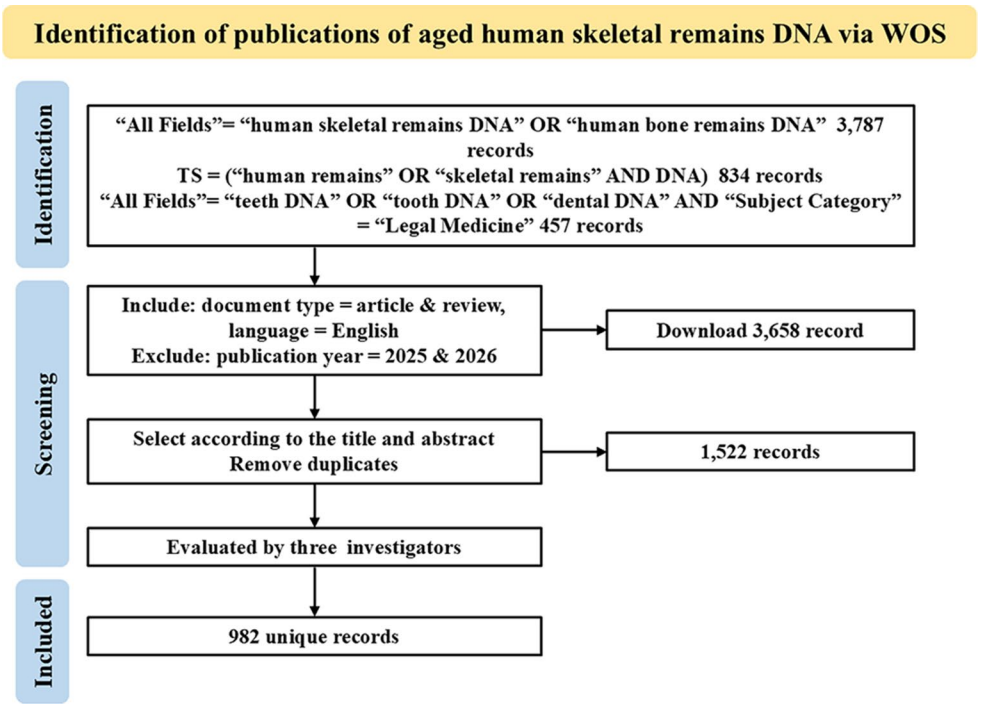

Fig. 2 Publications search strategy of aged human skeletal remains DNA

terms were retained in their full form; (4) Singular and plural forms were standardized to singular; for example, "teeth" was changed to "tooth". Additionally, author names were cleaned by formatting as "Last Name, First Name," with a comma separating the surname and given name, and initials capitalized. English author names were kept in full, while Chinese author names (in pinyin) were processed by removing hyphens. Country, region, and organization names were capitalized with initials, and abbreviations were expanded to their common full names—for example, "USA" was replaced with "United States." Regional names were further standardized to corresponding country names.

Data analysis and visualization

VOSviewer, a user-friendly bibliometric software, enables professional quantitative analysis and visualization of publications. Biblioshiny (RStudio v 2024.09.1) provides more comprehensive bibliometric metrics and functions. CiteSpace v.6.3.1.0 excels in detecting keyword bursts and performing advanced clustering analyses. This study integrates the complementary strengths of these three tools alongside other visualization methods to examine seven key indicators: (1) annual publication and citation trends analyzed by Biblioshiny (RStudio); (2) publication outputs by country and organization, and citation relationships between countries analyzed using VOSviewer, with national geographic visualization and citation chord diagrams generated by Scimago Graphica v1.0.47, and international collaborations explored via Biblioshiny (RStudio); (3) authorship networks of authors with more than four publications analyzed by VOSviewer using appropriate thresholds, complemented by publication timeline analyses conducted with Biblioshiny (RStudio); (4) journal source analysis performed with VOSviewer; (5) citation frequencies of individual articles examined using Biblioshiny (RStudio); (6) co-occurrence network analysis of author keywords with frequency thresholds conducted by VOSviewer, alongside professional keyword clustering and burst detection using CiteSpace; (7) three-field (sources-countries-author keywords) analysis performed with Biblioshiny (RStudio). The statistical results were visualized using Microsoft Excel (Office 2021).

To assess author impact, this study employed the H-Index, G-Index, and M-Index. The H-Index is defined as the maximum value H such that an author has published at least H articles, each cited at least times, serving as a widely accepted measure of academic influence. The G-Index complements the H-Index by incorporating the citation performance of highly cited publications, defined as the largest number G such that the top G articles have collectively received at least G^2 citations, thereby highlighting the impact of influential works. The M-Index adjusts for academic career length by dividing the H-Index by the number of years since the author's first publication. To evaluate international scientific collaboration, two indicators were applied: multiple country publications (MCP), representing articles authored by researchers from multiple countries, and single country publications (SCP), indicating those authored solely by researchers from a single country.

Result

The annual trends in growth and average citations of publications

This study included a total of 982 publications from 1989 to 2024, contributed by 4,486 authors affiliated with 1,493 organizations across 91 countries. These works appeared in 172 journals and included 1,983 unique author keywords alongside 25,510 cited references. Publication output was continuous and consistent from 1989 to 2024 without missing data, exhibiting an overall steady growth trend with an average annual growth rate of 10.36% (Fig. 3). The earliest publication included in this study is the pioneering 1989 *Nature* paper by Hagelberg et al., which overcame major technical barriers and established a methodological foundation for aDNA research [5]. Around the same period, Horai S et al. successfully amplified and sequenced the noncoding region of mtDNA from a human skull dated to approximately 6000 years BP [22]. Since 1989, publication output has shown a steady upward trajectory, rising from fewer than 10 articles per year in the early 1990 s to over 50 in recent years, with a peak of 72 in 2021 before declining to 51 in 2022. The most substantial annual increase (16 articles) occurred between 2018 and 2019, whereas 2022 showed a sharp decrease of 21 articles. Citation analysis revealed two distinct peaks of interest in early aDNA studies: the first in 1989, driven by the seminal work of Hagelberg E et al., and the second in 1991, corresponding to the study by Stoneking M et al., which pioneered the integration of PCR with sequence-specific oligonucleotide probes for analysis of mtDNA polymorphisms in skeletal remains [23]. Average citations per article reached a maximum of 166.50 in 1989, followed by 145.43 in 1991 and 119 in 1992. In subsequent years, average citations remained below 80, with a steady decline beginning in 2017 and reaching a low of 1.27 citations per article in 2024, a pattern likely attributable to the recency of publication (Supplementary Table S1).

Countries and organizations distribution

Countries

The number of publications, citation counts, total link strength (TLS), and average publication year (Avg. Pub. Year) were employed to evaluate the academic contribution and research activity of each country. TLS represents the cumulative strength of co-authorship or co-occurrence links between a given country and others, thereby reflecting its degree of international collaboration and centrality within the global research network. The Avg. Pub. Year denotes the mean publication year of articles originating from a specific

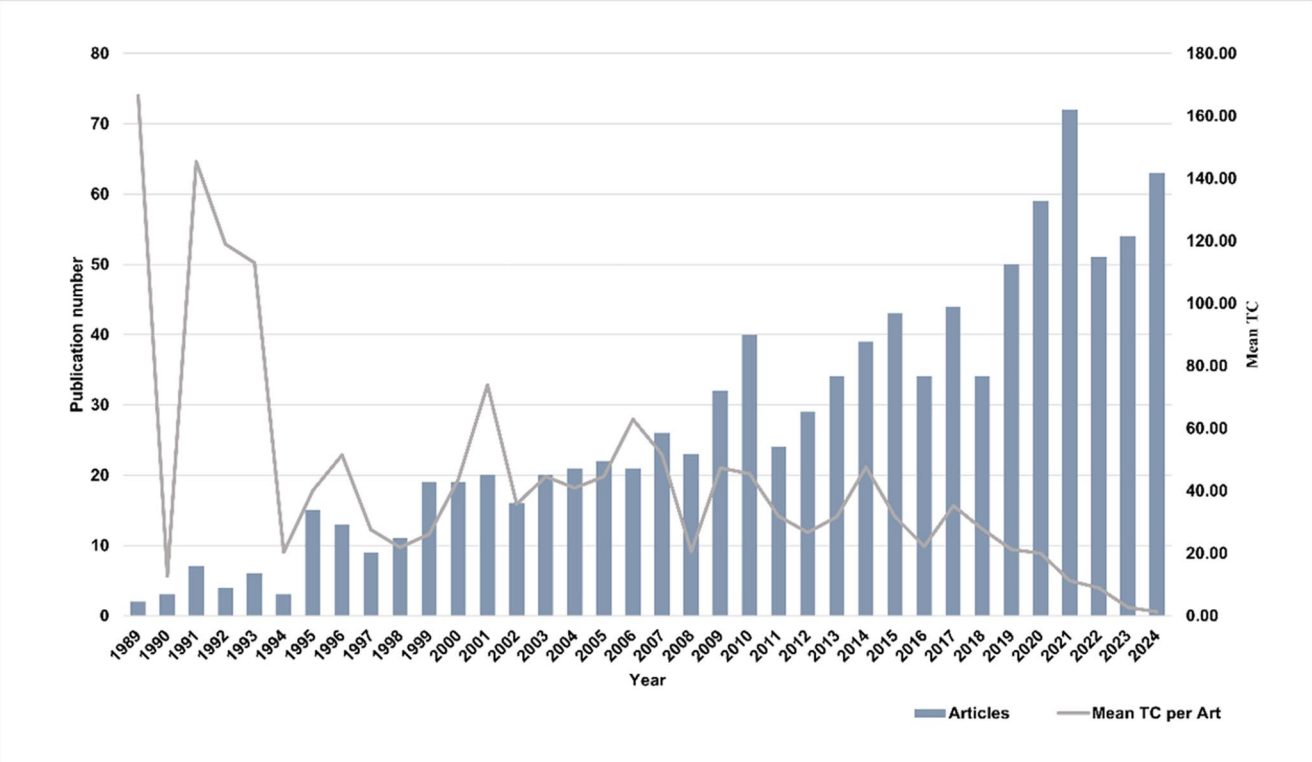

Fig. 3 Chronological distribution of publications and mean total citation per publication of aged human skeletal remains DNA. The bar chart shows the annual publication number. The gray line represents the trend of mean total citations per article (Mean TC per)

country and serves as an indicator of the recency and timeliness of its scientific output.

Among all contributing countries, only four produced more than 100 articles (Supplementary Table S2). The United States ranks highest in both publication number and citation count (302 publications and 11,818 citations), followed by Germany (179 publications, 8,361 citations), the United Kingdom (128 publications, 6,304 citations), and Italy (110 publications, 2,526 citations) (Supplementary Fig.S2 a). Five countries have publication counts between 50 and 100: Spain (86 publications, 3,821 citations), Australia (82 publications, 3,506 citations), France (68 publications, 3,023 citations), Japan (56 publications, 1,084 citations), and China (50 publications, 2,134 citations). All remaining countries contributed fewer than 50 publications each. The top five countries in terms of TLS are the United States (491), Germany (458), the United Kingdom (342), Spain (219), and Australia (219), indicating their prominent roles in global collaborative networks and sustained engagement in international forensic genetics research.

The temporal publication characteristics of countries (Supplementary Fig.S2 b) and the average publication year (Supplementary Table S2) indicate Japan (2008.30), Croatia (2011.06), the United States (2013.17), South Korea (2013.14), and the United Kingdom (2013.63) exhibit the darkest purple nodes, reflecting a longer-standing presence in aged human skeletal remains DNA research. In contrast, yellow nodes represent more recent contributors, such as Slovenia (2020.42), Austria (2018.34), Poland (2018.30), Switzerland (2018.12), and Australia (2018.04) showing relatively high recent activity. Geographic visualization developed by Scimago Graphica reveals that the highest publication outputs in this field are mainly concentrated in the Americas and Europe, especially in developed countries (Supplementary Fig.S2 c).

Research collaboration among corresponding authors' countries was measured by MCP and SCP (Supplementary Table S3). Austria (86.7%), Sweden (71.4%), and Denmark (70.6%) demonstrated notably high MCP ratios, indicative of extensive international collaboration, while Japan (10.6%), India (15.4%), and Slovenia (20.0%) show relatively low MCP (Supplementary Fig.S3). Although the United States published 197 articles, its low MCP proportion reflects robust independent research capacity. Canada showed a nearly balanced profile, with equal MCP and SCP proportions (50.0% each), reflecting a well-distributed mix of domestic and international collaborations.

Organizations

The Max Planck Institute for Evolutionary Anthropology leads the field with 57 publications, accumulating 4,624 citations and a TLS of 196, underscoring its dominant position in research output, academic influence, and international collaboration (Supplementary Table S4). The University of Ljubljana (41 publications, 345 citations) and the University of Copenhagen (33 publications, 2,158 citations) also demonstrated outstanding productivity. Within the international collaboration network, besides the Max Planck Institute for Evolutionary Anthropology, which leads in institutional links, the Max Planck Institute for the Science of Human History, Harvard University, University of Copenhagen, and Russian Academy of Sciences also maintain active collaborations. In contrast, the University of Tennessee, the University of Tokyo, the University of Göttingen, Jilin University, and the University of Florence exhibited lower link strengths, highlighting the necessity to leverage harnessing additional resources and innovative capabilities (Fig. 4). Temporal analysis of publication activity reveals that organizations including the Federal Bureau of Investigation, the University of Oslo and the University of Barcelona—represented by the darkest purple nodes—have been seminal early contributors. In contrast, the University of Ljubljana, Harvard Medical School, and the Max Planck Institute for the Science of Human History, which are represented as yellow nodes, have been particularly active in recent years (Fig. 4). At the national level, Germany, home to the Max Planck Society institutes (Evolutionary Anthropology and Science of Human History), leads comprehensively in both academic impact and international collaboration intensity.

Author and co-author relationship

Publication count, citation frequency, H-Index, G-Index, and M-Index assessed author productivity. TLS measured collaboration intensity, and Avg. Pub. Year indicated academic activity. Publication data for the top 20 authors by output are summarized in Supplementary Table S5.

Authors' academic productivity is concentrated among a few prolific researchers (Supplementary Fig.S4 a). Pajnič, Irena Zupanič from the University of Ljubljana, Slovenia, is the most prolific author (39 publications, 341 citations), followed by Pääbo, Svante from the Max Planck Institute for Evolutionary Anthropology, Germany (24 publications, 3,889 citations) and Krause, Johannes also from the Max Planck Institute for Evolutionary Anthropology (23 publications, 1823 citations) (Supplementary Table S5). Notably, the author with the highest publications differs from the most cited author. To address this distinction, the H-Index, G-Index, and M-Index were introduced to evaluate academic productivity (Table 1). Results indicate

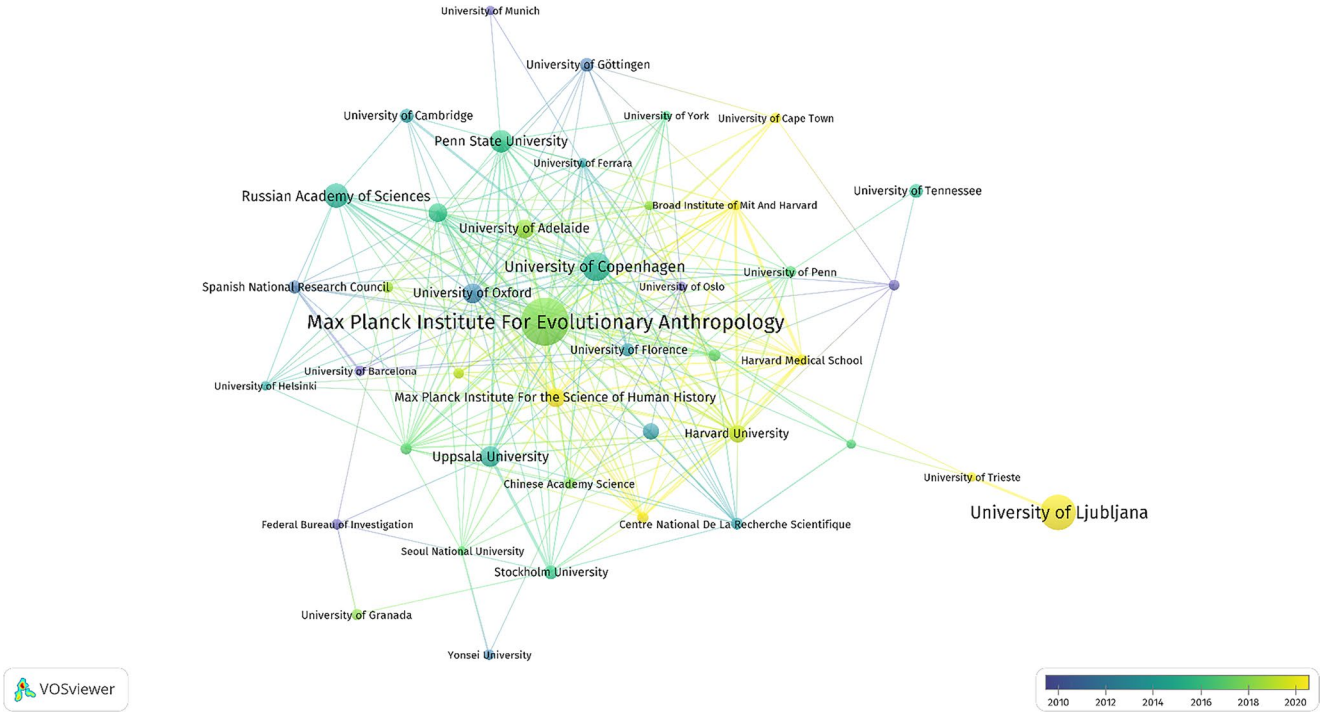

Fig. 4 Network visualization of organizations with at least 10 publications on aged human skeletal remains DNA. Each node represents an organization, node size indicates publication count, links indicate collaborations, and node color reflects the temporal trend of publications

Table 1 H-Index, G-Index and M-Index of authors on aged human skeletal remains DNA

Author	H-Index	G-Index	M-Index
Pääbo, Svante	34	56	1.42
Willerslev, Eske	25	41	1.39
Krause, Johannes	24	40	1.41
Budowle, Bruce	23	36	0.7
Ludes, Bertrand	16	25	0.7
Meyer, Matthias	15	24	1.36
Pajnič, Irena Zupanič	10	15	0.91
Županc, Tomaz	9	13	0.82
Caramelli, David	8	12	0.57
Hummel, Susanne	7	10	0.23

that Pääbo, Svante has the highest H-Index (34), reflecting the greatest foundational academic influence, followed by Willerslev, Eske (25) and Krause, Johannes (24). The G-Index is highest for Pääbo, Svante (56), Willerslev, Eske (41), and Krause, Johannes (40), demonstrating superior overall citation quality and impact. Pääbo, Svante (1.42) exhibits the highest M-Index, followed by Krause, Johannes (1.41), and Willerslev, Eske (1.39), underscoring their consistent productivity and significant scientific contributions.

Regarding academic collaboration, Krause, Johannes has the highest TLS (115), indicating his leading role in scholarly interactions, followed by Meyer, Matthias (105) and Pääbo, Svante (101) (Supplementary Table S5). In inter-team collaborations, the purple cluster centered on Pääbo, Svante (Supplementary Fig.S4 b) shows the most extensive connections to other nodes, highlighting a broad academic network. The author co-citation network (Supplementary Fig.S4 a) reveals five distinct clusters: aDNA and archaeogenetics (Willerslev, Eske; red), forensic genetics (Budowle, Bruce; green), East Asian population structure and evolution (Zhou Hui; yellow), Denisovan and Neanderthal study (Pääbo, Svante; purple), and identification of war remains and population structure in Eastern Europe (Pajnič, Irena Zupanič; blue). Pääbo, Svante is represented by the largest and most central node, highlighting his pivotal role in interdisciplinary collaboration and substantial impact on the field.

The temporal distribution of author publications illustrates both individual research trajectories and the spatio-temporal development of global studies on aged human skeletal remains DNA (Fig. 5). Pajnič, Irena Zupanič, who commenced her contributions relatively recently around 2013, ranks first in publication output due to her high productivity. Pääbo, Svante, ranked second, published extensively, and gained broad recognition as early as the 1990s. Budowle, Bruce has been cited since 1991, is among the field's senior figures. Meyer, Matthias began publishing articles in 2014, with relatively high average annual citations (indicated by darker dots). Leskovar, Tamara has been active in publishing in recent years (Supplementary Table S6).

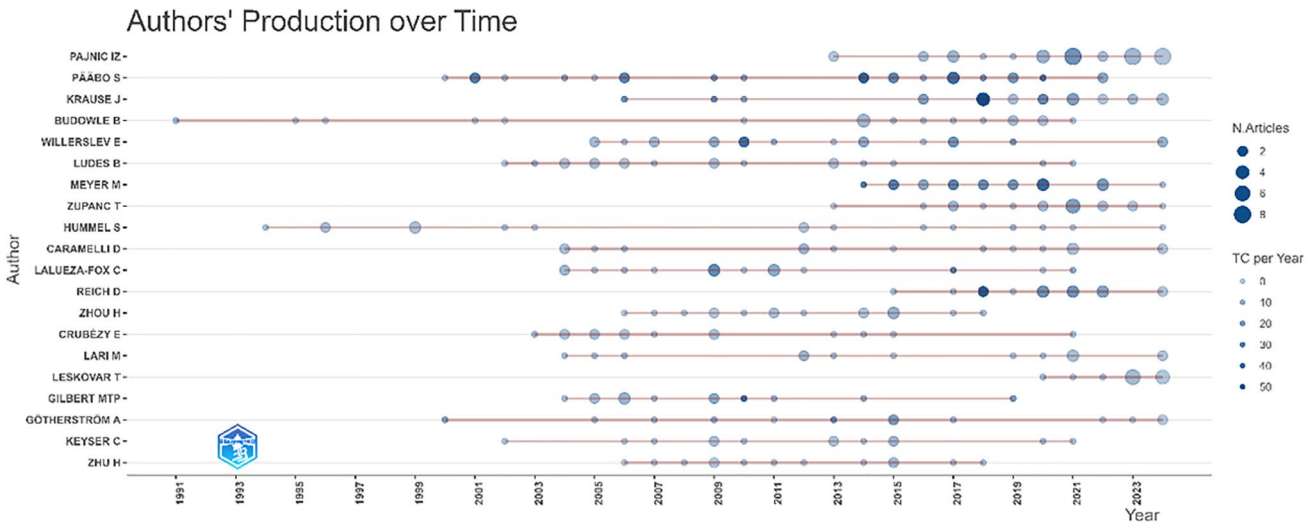

Fig. 5 Contribution of the top 20 authors over different years (red lines) by Biblioshiny (RStudio). The size of the dots indicates the number of publications (N. Articles) over different years, and the color of the dots (light to dark) indicates the total citations per year (TC per year)

Sources of publications

The journals with the highest number of publications in the field of aged human skeletal remains DNA include *Forensic Science International*, *Journal of Forensic Sciences*, *Forensic Science International: Genetics*, *International Journal of Legal Medicine*, *and American Journal of Physical Anthropology* (Supplementary Table S7). The most cited journals are *Nature* (16 publications, 2,687 citations), *Forensic Science International: Genetics* (80 publications, 2,377 citations), *Journal of Forensic Sciences* (81 publications, 2,342 citations), *Forensic Science International* (89 publications, 2,081 citations), and *Proceedings of the National Academy of Sciences of the United States of America* (24 publications, 1,678 citations).

The *Journal of Forensic Sciences* began publishing articles related to aged human skeletal remains DNA in *1991* (Fig. 6). *Forensic Science International*, *International Journal of Legal Medicine*, *American Journal of Physical Anthropology*, and *Journal of Archaeological Science* started to include such articles between 1990 and 1996, while *Scientific Reports* began relatively late, in 2015. The *Journal of Forensic Sciences* held the leading publication count until 2021when *Forensic Science International* surpassed it. *Forensic Science International: Genetics* started publishing relevant articles in 2007 and has experienced the fastest growth. Since 2019, *Forensic Science International*, *Forensic Science International: Genetics*, and the *Journal of Forensic Sciences* have published significantly more articles than other journals.

Document and citation relationship

Citation counts reflect the quality and recognition of the articles in the field. Table 2 lists the top 20 cited articles, with the article Hofreiter M, 2001, Nat Rev Genet leading at 666 citations, followed by Rasmussen M, 2010, Nature (559), and Noonan Jp, 2006, Science (414). Rasmussen M, 2010, Nature also has the highest average annual citations (34.94), ahead of Vernot B, 2014, Science (30.50) and Sehlebusch Cm, 2017, Science (29.89). Publications in high-impact journals such as *Nature* and *Science* generally achieve greater citation frequencies. The article Hofreiter M, 2001, Nat Rev Genet is a seminal review addressing DNA degradation and preservation challenges, outlining key technical issues in aDNA research [24].

Co-occurrence of author keywords

Keyword analysis provides insight into research hotspots, emerging trends, and academic influence while also facilitating interdisciplinary integration. “Co-occurrence” refers to the frequency with which keywords appear together across multiple publications. Effective titles, well-structured abstracts, and precise keyword selection are critical for enhancing article visibility and citation potential. Co-occurrence analysis quantitatively evaluates keyword frequencies to uncover thematic relationships and knowledge structures within the field. In this study, author keywords occurring at least four times were included, with only 98 out of 1,967 meeting this threshold.

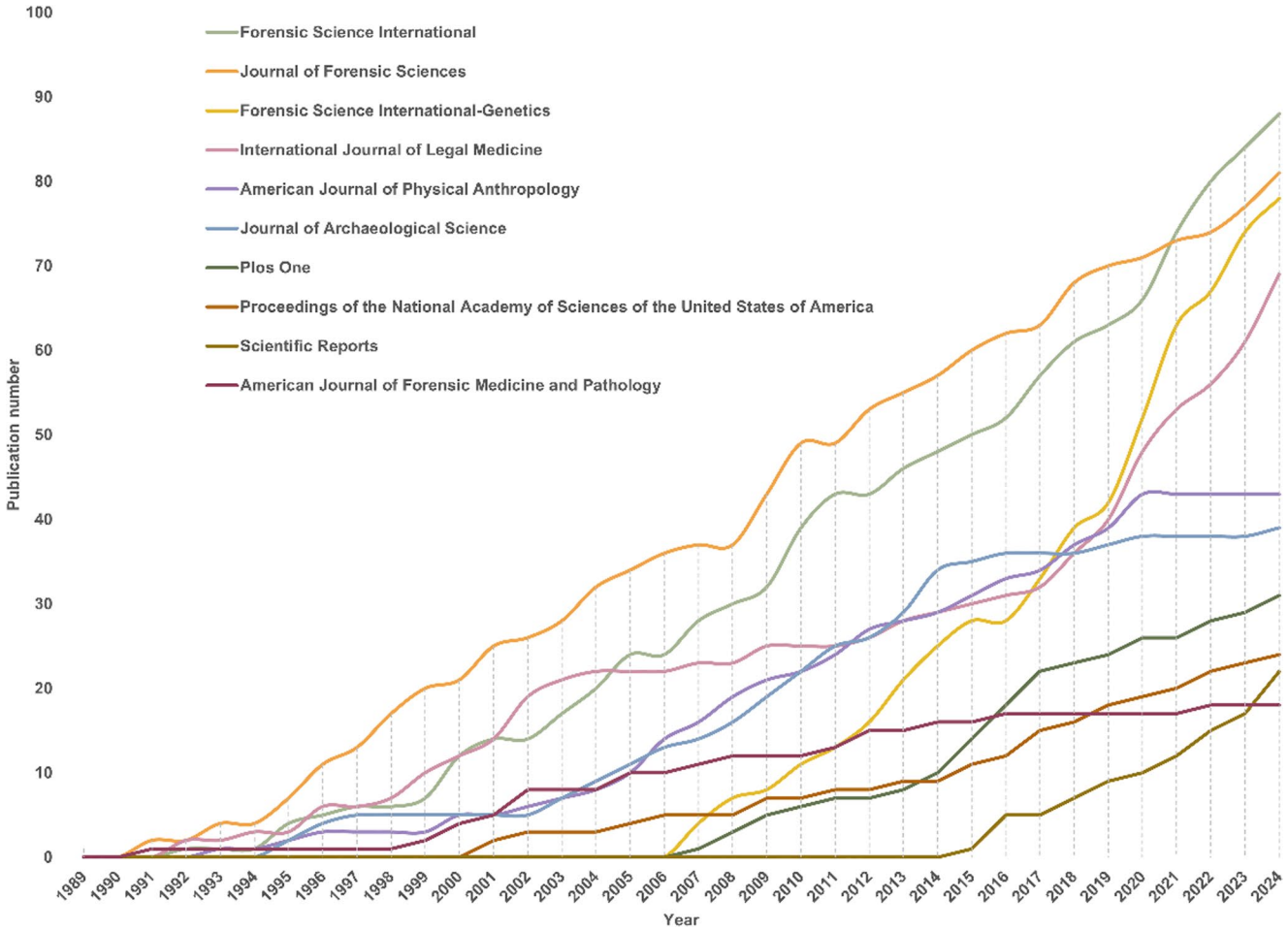

Fig. 6 Annual publication trends of the top 10 journals in aged human skeletal remains DNA study from 1989 to 2024. The x-axis shows years; the y-axis shows the number of publications. Each color represents a journal

The results (Supplementary Table S8) show that only five author keywords appeared more than 100 times. The keywords "aDNA" had the highest frequency (256), followed by "human skeletal remains" (175), "mtDNA" (171), "forensic science" (137), and "human identification" (113). The keywords with the highest TLS were "human skeletal remains" (489), followed by "forensic science" (438), "mtDNA" (403), "aDNA" (396), and "human identification" (293).

A visual analysis of 93 author keywords (each appearing at least four times) is presented in Supplementary Fig.S5 and Fig.S6. In Fig.S4, "aDNA," "human skeletal remains," "mtDNA," "forensic science," "human identification," and "DNA" appear as dominant nodes. Temporal color gradients reveal the rising trends of different genetic markers, technologies, and emerging research areas. "PCR" and "sequencing" are shown in the darkest purple, reflecting their long-standing use in the field, whereas "MPS" and "ATR-FTIR spectroscopy" are highlighted in bright yellow, indicating their more recent application. For genetic markers, the transition from purple to yellow across "microsatellites," "mtDNA," "STR," and "SNP" illustrates their developmental trajectory. Similar temporal patterns are observed for research topics such as "sex identification," "kinship analysis," "DNA preservation," and "Second World War." Overall, these temporal trends are consistent with the findings of our literature review and the milestone summary (Fig. 1).

CiteSpace clustering identified nine major thematic groups: #0 aDNA, #1 forensic science, #2 mtDNA, #3 STR typing, #4 DNA extraction, #5 PCR. Clusters involving mtDNA, DNA extraction, forensic science, and PCR showed notable overlaps (Supplementary Fig.S6 a), reflecting the integrative and evolving nature of the field. Citation burst analysis shows an early and sustained burst for "PCR" starting in 1998 and "DNA typing" in 1996, while "MPS" exhibited a strong burst beginning in 2019 with continued impact (Supplementary Fig.S6 b).

Table 2 Top 20 articles in the field of aged human skeletal remains DNA with the highest number of citations

Document	Total Citations	TC per Year	DOI
Hofreiter M, 2001, Nat Rev Genet	666	26.64	https://doi.org/10.1038/35072071
Rasmussen M, 2010, Nature	559	34.94	https://doi.org/10.1038/nature08835
Noonan Jp, 2006, Science	414	20.70	https://doi.org/10.1126/science.1131412
Briggs Aw, 2009, Science	368	21.65	https://doi.org/10.1126/science.1174462
Vernot B, 2014, Science	366	30.50	https://doi.org/10.1126/science.1245938
Meyer M, 2014, Nature	348	29.00	https://doi.org/10.1038/nature12788
Ovchinnikov Iv, 2000, Nature	330	12.69	https://doi.org/10.1038/35006625
Stoneking M, 1991, Am J Hum Genet	318	9.09	-
Skoglund P, 2013, J Archaeol Sci	282	21.69	https://doi.org/10.1016/j.jas.2013.07.004
Serre D, 2004, Plos Biol	273	12.41	https://doi.org/10.1371/journal.pbio.0020057
Sehlebusch Cm, 2017, Science	269	29.89	https://doi.org/10.1126/science.aao6266
Hagelberg E, 1989, Nature	246	6.65	https://doi.org/10.1038/342485a0
Prinz M, 2007, Forensic Sci Int-Gen	245	12.89	https://doi.org/10.1016/j.fsigen.2006.10.003
Alaeddini R, 2010, Forensic Sci Int-Gen	240	15.00	https://doi.org/10.1016/j.fsigen.2009.09.007
Kemp Bm, 2005, Forensic Sci Int	236	11.24	https://doi.org/10.1016/j.forsciint.2004.11.017
Slon V, 2017, Science	234	26.00	https://doi.org/10.1126/science.aam9695
Hagelberg E, 1991, Nature	224	6.40	https://doi.org/10.1038/352427a0
Holland Mm, 1993, J Forensic Sci	219	6.64	-
Loreille Om, 2007, Forensic Sci Int-Gen	215	11.32	https://doi.org/10.1016/j.fsigen.2007.02.006
Posth C, 2018, Cell	205	25.63	https://doi.org/10.1016/j.cell.2018.10.027

Three-field plot analysis representing sources-countries-authors keywords

Three-field analysis (Biblioshiny, RStudio) visualizes the relationships among journals, countries, and author keywords (Fig. 7). Results show the keyword "aDNA" connected strongly with countries such as the United States, Italy, and Germany, as well as journals like *Forensic Science International*, *Forensic Science International: Genetics*, *and Journal of Forensic Sciences*. The term "mtDNA", the second most prevalent keyword, is associated strongly with countries such as the United States, United Kingdom, and Italy, and journals such as the *Journal of Forensic Sciences*, *Forensic Science International: Genetics*, and *American Journal of Physical Anthropology*. Other keywords like forensic science, human skeletal remains, and DNA also link to specific journals and countries. Journals on ancient human skeletal DNA mainly concentrate on forensic science, forensic genetics, and forensic anthropology. The flow from journals to author keywords shows these journals emphasize DNA and skeletal analysis. Countries-author keywords links reveal the United States and Italy dominate aDNA and DNA research, while France and Germany focus more on forensic science and skeletal remains.

Discussion

Temporal trends and citation impact

The field of aged human skeletal DNA—an increasingly significant subdomain within forensic genetics—has progressed from research methodologies based on PCR and Sanger sequencing to high-throughput and highly accurate genetic analysis between 1989 and 2024 (Fig. 1). These phases reflect a general upward trajectory in publication volume, driven primarily by technological innovations and interdisciplinary integration. A notable inflection point occurred around 1995, when annual publications exceeded ten. This surge was catalyzed by the landmark 1994 application of mtDNA analysis to the remains of Tsar Nicholas II, which successfully integrated aDNA methodologies into forensic science and introduced advanced silica-based DNA extraction protocols [25]. In 2003, the identification of victims from the World Trade Center attacks used nuclear STR/miniSTRs, mtDNA, and sex markers, combined with statistical methods such as random match probability and likelihood ratios. This strategy achieved high-precision results and set a benchmark for skeletal DNA analysis in mass-disaster context [26]. By 2010, the draft genome of the Neanderthal, followed by a high-quality version in 2014, established paleogenomics as a distinct field and deepened the molecular understanding of archaic hominins [27]. Around the same time, forensic utility was further underscored in 2014 with the identification of Richard III via complete mtGenomes sequencing using MPS and Y-chromosome analysis, in conjunction with archaeological evidence [4]. In 2018, the Golden State Killer case highlighted the utility of degraded DNA and genetic genealogy, providing valuable insights for skeletal DNA research [28]. In addition, technological advances have facilitated major international efforts, including DNA identification from

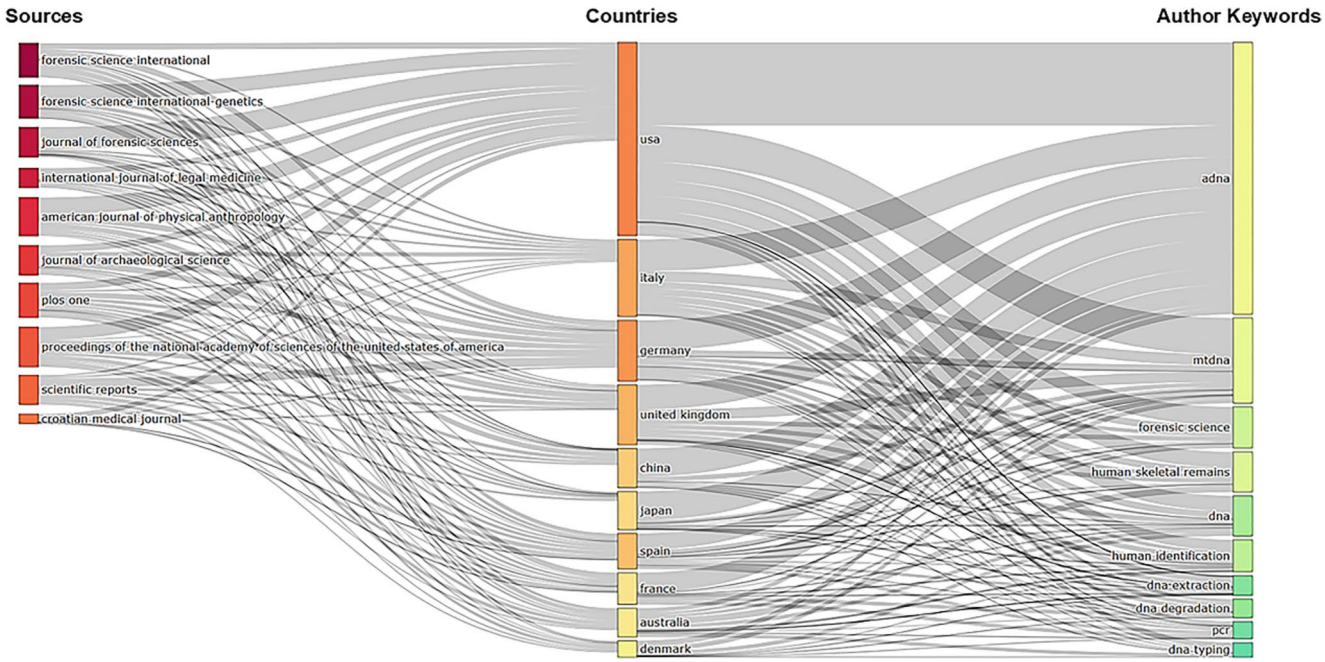

Fig. 7 Three-field plot of journals, countries, and author keywords. The boxes and lines illustrate the associations among the three variables, with box height and line thickness representing the strength of these associations

20th-century war remains. These efforts have promoted publications addressing phenotypic traits, kinships, and geographic origins, while supporting the creation of genetic databases [2]. Publication output peaked in 2021 and key advancements may include: Irena Zupanic's comparative analysis of DNA extraction success rates across different skeletal elements (metacarpals, ribs, and vertebrae) [29–31]. Afra K's exploration of cranial phenotype-SNP correlations for facial reconstruction [32]. These developments reflect dual progress in enhancing DNA recovery techniques while advancing standardization in the field.

As for citation, both early and recent publications are affected by the time elapsed since publication and the total number of related articles. The high citation rate of early studies largely reflects their temporal advantage and the cumulative nature of scientific knowledge, while citation rates for recent years (2017~2024) likely reflect a citation lag rather than reduced impact, as new publications have limited time to accrue citations and may not yet be fully indexed.

The contributions of countries and organizations

Research on aged human skeletal DNA has been predominated by institutions in Europe and North America. Key contributors include the University of Ljubljana (Slovenia), Max Planck Institute for the Science of Human History (Germany) and the Harvard University (United States). Several contextual factors may explain the concentration of research capacity in Europe and North America. These include substantial investments in basic science, access to rich archaeological resources (e.g., Neanderthal and Paleolithic sites), permissive ethical regulations regarding human remains, and strong infrastructure supporting interdisciplinary and international collaboration. For example, situated at the crossroads of Central and Southern Europe, the University of Ljubljana leverages the region's rich historical human remains to study DNA preservation under diverse conditions, optimize sample selection, enhance extraction efficiency and quality, and develop reliable DNA tools for human remains identification, resulting in a substantial body of recent research and practical applications in forensic and archaeological contexts. The Max Planck Institute for the Science of Human History integrates genetics, archaeology, and anthropology, forming a robust research platform that provides multifaceted support for ancient human DNA studies.

In terms of international collaboration, research in the United States is largely driven by domestic resources, reflecting strong independent research capacity and a comparatively low reliance on international collaboration. In contrast, countries such as Japan, Slovenia, and China tend to prioritize internal collaborations, thereby fostering more localized research networks. Research in these countries is often concentrated in forensic science, particularly in the study and application of human skeletal remains, with relatively limited engagement in international collaboration. Expanding international collaborations would

facilitate reciprocal knowledge and accelerate methodological innovation.

The impact of authors and journals

Core authors in the field include Pääbo, Svante; Willerslev, Eske; Krause, Johannes; and Pajnič, Irena Zupanič, with research spanning archaeology and forensic genetics. Pääbo, Svante, a pioneering figure in paleogenomics, led the sequencing of Neanderthal and Denisovan genomes, uncovering previously unknown admixture events with modern humans [27, 33]. Along with Meyer and Krause, he developed fundamental aDNA methodologies that remain central to aged skeletal DNA research. Willerslev E's research consistently focuses on extracting ancient DNA from permafrost and cave sediments, employing MPS and hybridization capture technologies to obtain more complete paleogenomic information, thereby providing critical technical support for the field [34]. Pajnič, Irena Zupanič, despite a relatively modest H-index (10) and G-index (15), is the most prolific author in this domain. Her work focuses on practical forensic applications of aDNA in post-conflict and historical contexts (e.g., the Balkans and WWII), including kinship analysis and phenotypic prediction. Her case-driven contributions demonstrate the field's translational value [35, 36].

According to Bradford's law, research output is concentrated in core journals such as *Forensic Science International*, *Journal of Forensic Sciences*, *Forensic Science International: Genetics*, *International Journal of Legal Medicine*, and *American Journal of Physical Anthropology*, which together publish approximately 37% of articles in the field. Notably, the prominence of forensic journals post-2019 reflects the maturation and consolidation of skeletal DNA research within forensic science.

High-frequency keywords and disciplinary characteristics

The most frequently occurring author keywords—aDNA, human skeletal remains, mtDNA, forensic science, and human identification—illustrate a strong methodological and conceptual alignment with aDNA research. Since the first successful extraction of mtDNA from a museum-preserved Quagga specimen in 1984 [37], the field of aDNA has evolved across multiple disciplines, including anthropology, archaeology, microbiology, and forensic genetics [38]. The aDNA methodologies, which emphasize contamination control and damage-tolerant extraction, have proven superior for handling degraded biological materials [15]. Their integration into forensic workflows is increasingly recommended. Hofreiter et al. [39] emphasized best practices for applying aDNA methods in forensics, from sample selection to bioinformatic processing, particularly for MPS. However, forensic applications demand higher standards of reproducibility, interpretability, and legal admissibility. This necessitates further validation of aDNA-informed protocols.

Keyword unification during data cleaning (e.g., consolidating synonyms under "human skeletal remains") may have influenced frequency rankings. Nonetheless, co-occurrence analysis further indicates that individual identification remains the field's core focus. Supplementary techniques such as stable isotope analysis and skeletal morphology offer essential context when genetic data are unavailable or insufficient [40].

Genetic markers and technological advances

Among genetic markers, mtDNA is the most frequently employed in early publications due to its high copy number, resilience to degradation, low mutation rate, and maternal inheritance [41]. Its utility was famously validated during the Romanov family identification [25]. The analysis of degraded DNA in skeletal remains has been significantly enhanced by the use of SNP markers. The transition from STRs to SNPs is primarily due to the superior performance of SNPs with degraded DNA, as demonstrated in the identification of human remains from Jeju Island in 2020 [19]. Concurrently, MPS-based targeted SNP analysis has gained widespread adoption for such samples. This method leverages the advantageous properties of SNPs, including high genomic abundance, short amplicon length, and low mutation rate, thus supporting a wide range of forensic applications [42]. The aSTRs continue to be a forensic gold standard, but their larger amplicons and susceptibility to degradation constrain their utility in aged remains. In contrast, Y-chromosome markers (Y-STRs and Y-SNPs) remain highly valuable for male-lineage tracing and mass grave investigations. X-STRs are gaining traction in complex kinship analyses due to their unique inheritance properties [2]. Microhaplotypes (MHs)—clusters of tightly linked SNPs—combine the advantages of both STRs and SNPs, while also overcoming their limitations, including no stutter, no amplification bias, short fragments, low mutation rate, low recombination rate, large number, wide distribution, and high polymorphism. Additionally, some MHs contain identity, ancestry, phenotype, and other information and thus have great value in areas such as personal identification, paternity testing, DNA mixture and degradation analysis, ancestry inference, and phenotype prediction, thereby attracting the attention of forensic researchers [43, 44]. They have already been incorporated into some MPS commercial kits (e.g., the ForenSeq Signature panel) and have been applied in the identification of war remains and missing persons [45, 46].

At the technical level, MPS represents the most transformative technological advancement in the field. It allows for the simultaneous detection of multiple marker types, making it ideal for comprehensive analyses of degraded samples. Hybridization capture has enabled the recovery of complete mtGenomes and informative SNPs from compromised remains [47]. A notable case is the study by P. Du et al. [9], who used over 1 million SNPs, 3D cranial reconstruction, and phenotypic prediction to generate a full genomic and morphological profile of Emperor Yuwen Yong. Such integrative analyses offer a model for future phenotype-based identifications. The application of artificial intelligence (AI) and machine learning (ML) are still being explored vigorously. For example, M. Ferrando-Bernal et al. highlighted the potential of AI to enhance phenotypic prediction from aDNA, underscoring future opportunities to expand inference beyond pigmentation to more complex traits and health predispositions [48]. Jiao M et al. introduced a novel framework called "Diffface" for reconstructing 3D facial morphology from DNA, which significantly enhanced reconstruction accuracy and reliability [49]. This approach holds considerable promise for reconstructing 3D facial features from skeletal remains DNA.

Persistent challenges in the field

Despite significant advances, persistent challenges remain in the study of aged skeletal DNA: (1) Variable DNA preservation. Influenced by intrinsic factors (e.g., bones' chemical composition, morphology, structure, density) and extrinsic factors (e.g., temperature, humidity, pH, organic matter, groundwater, microbial activity, biotic influences), DNA degradation patterns remain poorly understood [50]. (2) Prescreening limitations. Current assessment relies heavily on DNA quantification, which is time-consuming and unsuitable for extremely degraded or limited samples. Alternatives like Fourier transform infrared spectroscopy (FTIR) have been proposed for improved prescreening [51, 52]. (3) Contamination and degradation. Both remain significant barriers to accurate genotyping, necessitating stricter quality control protocols. (4) Limited accuracy in phenotypic prediction. Current tools lack precision, especially in degraded samples, calling for more robust models and validation. For example, systems such as HIrisPlex and HIrisPlex-S, while promising, have yet to demonstrate consistent accuracy when applied to degraded skeletal samples. This limitation arises because skeletal DNA is highly degraded, present only in small quantities, and often coexists with contaminants and PCR inhibitors, frequently leading to locus dropout or genotyping errors [17]. (5) Need for population-specific databases. Accurate inference requires extensive allele frequency data for diverse populations. (6) Multidisciplinary integration. Combining AI with modern anthropological and archaeological tools could enhance craniofacial reconstruction and phenotypic inference.

Study limitations and future directions

This bibliometric analysis employed VOSviewer, Biblioshiny (RStudio), CiteSpace, and Scimago Graphica to analyze and visualize research trends in aged skeletal DNA. Based on preliminary evaluation, VOSviewer and Biblioshiny (RStudio) were chosen for their superior compatibility with the dataset, though methodological differences between the tools may affect consistency. Limitations include the exclusion of non-English publications and reliance solely on WoS, potentially omitting relevant data from other repositories. VOSviewer's lack of support for qualitative content analysis and its sensitivity to threshold settings may have introduced additional biases. Despite these constraints, the study offers a comprehensive overview of global trends and key developments in the field of aged human skeletal remains DNA. Future work should consider integrating multiple databases, refining keyword strategies, employing mixed-method approaches, and conducting sensitivity analyses to improve result robustness and inclusivity.

Conclusion

This study presents a comprehensive overview of global research trends, technological advancements, and patterns of collaboration in the field of DNA from aged human skeletal remains. The growing volume of publications in recent years reflects increasing international recognition of the scientific and forensic value of this domain. Nevertheless, the analysis of aged skeletal DNA remains a relatively nascent area within forensic science, necessitating the continued refinement of aDNA methodologies to meet the rigorous standards of forensic practice. Research output remains predominantly concentrated in developed countries—particularly the United States, Germany, Italy and the United Kingdom—where well-established institutional infrastructures and funding frameworks continue to drive innovation and methodological progress. To promote more equitable and sustainable development, it is essential to strengthen international collaborations. Such partnerships can facilitate the global dissemination of technologies, foster the standardization of analytical protocols, and enhance the reproducibility and applicability of findings across diverse forensic contexts. The forensic value of DNA from aged skeletal remains extends beyond the identification of historical figures, war casualties, disaster victims, and missing persons. It holds considerable potential in resolving cold

cases, thereby advancing the pursuit of justice and reinforcing public trust in forensic science. In this regard, the present study may serve as a valuable reference for shaping strategic priorities and fostering a more globally integrated forensic framework for future forensic genetic research.

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Declarations

Conflict of interest The authors have declared no conflicts of interest.

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