Data mining of DNA sequences submitted by Peruvian institutions to public genetic databases

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Title

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Título

Minería de datos de secuencias de DNA enviadas a bases de datos genéticas públicas por instituciones peruanas

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Contribution of each author / Información sobre la contribución

PER participated in the conceptualization, data analysis, writing and review of the manuscript. CC-V participated in data analysis and writing of the manuscript
PER participó en la concepción, análisis de datos, escritura y revisión del manuscrito
CC-V participó en el análisis de los datos y la escritura del manuscrito.

Abstract

Peru is one of the most biodiverse countries in the world. Genetic diversity is an important component of biodiversity, and it is crucial for current efforts to protect and sustainably manage several organisms and habitats. As far as we know, there is only one work describing Peruvian genetic information stored in public databases. We aimed to update this previous work searching in four public databases that stored sequencing information: Nucleotide, BioProject, PATRIC), BOLD. With this information we comment on the contribution of Peruvian institutions during recent years. In Nucleotide, the largest database, Bacteria are the most sequenced organisms by Peruvian institutions (70.60%), pathogenic bacteria such as *Pasteurella multocida*, *Neisseria meningitidis*, and *Vibrio parahaemolyticus* were the most abundant. We found no sequence records from the Archaea domain. In BioProject, the most common sequence belongs to *Salmonella enterica* subsp. enterica serovar Infantis. In PATRIC, a database for pathogenic agents, *Mycobacterium tuberculosis* and *Yersinia pestis* had the highest number of entries. Finally, in BOLD, exclusively Eukaryotic database, Chordata (Aves and Actinopterygii), Angiospermae, and Arthropoda (Insecta, and Arachnida) were the most frequent records. Our results would indicate research preferences of Peruvian institutions, focusing on infectious diseases. Although there has been a significant increase of DNA information submitted by Peruvian institutions since the last report, the genetic diversity reflected in these databases remains inconsistent with the diversity in the country. More efforts must be made to know obtain genetic information from more underestimated taxonomic groups and to promote more genetic research in regional Peruvian institutions.

TITLE

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Introduction

Peru is one of the most biodiverse countries in the world. Current estimates of species richness showed the occurrence of numerous species, such as plants (20 533), vertebrates (5 738), and arthropods (30 547) (MINAM, 2019). There are no current records of the extant Peruvian diversity of bacteria or archaea, though. Conservation and management of this vast biodiversity requires different approaches considering ecological and systematic knowledge, and, recently, information about genetic diversity (Norena et al. 2018). This information is essential not only for conservation and taxonomic studies but also for bioprospecting novel compounds (Sekurova et al 2019) or for breeding programs assisted by genetic markers (Assefa et al. 2019, Mrode 2019).

Bioinformatics, the discipline that uses computational approaches to answer biological questions, has become essential to analyze genetic and genomic information (Baxevanis and Ouellette 2005). Especially, to study large and complex DNA datasets obtained via next-generation sequencing (NGS), to discover new genes, functions, pathways, and molecular interactions. Most of this information is available in online public databases. For instance, GenBank (https://www.ncbi.nlm.nih.gov/genbank), at the National Center for Biotechnology Information from the US National Institute of Health, currently possess 216,531,829 sequences, being one of the largest source worldwide. Other databases such as the Barcode of Life Data System (https://www.boldsystems.org), 2,869,168 sequences, are more specific, providing information from specific eukaryotic genes.

The representativeness of Latin American data in genome projects and public databases has been discussed since the beginning of the genomic era (Ramírez et al. 2002). The information stored in databases has become essential to study the genetic diversity of indigenous peoples (Harris et al. 2018), human diseases (Norris et al. 2018), and regional biotechnological and agro-industrial challenges (Sasson & Malpica 2018, Wang et al. 2017).

The most recent assessment of the Peruvian genetic data in public databases was done by Norena et al. (2018). The authors estimated 645 753 sequences in the NCBI Nucleotide database associated with the term "Peru", from which 6 522 (1.01%) were published by national institutions. We updated the amount of sequencing data of the Peruvian biodiversity submitted by national institutions to four main public genetic databases: NCBI BioProject, a

database that comprises multiple collections of biological data related to a single genomic sequencing initiative. NCBI Nucleotide that comprises GenBank and related databases. The exclusively bacterial database Pathosystems Resource Integration Center (PATRIC) which provides sequencing, protein-protein interactions and transcriptomic data (Wattam et al. 2017), and the Barcode of Life Data System (Ratnasingham & Hebert, 2007), a DNA barcode database, focused in single markers, namely, the cytochrome oxidase I (COI) gene for animals, internal transcribed spacers (ITS) for fungi, and the ribulose-bisphosphate carboxylase (rbcL) and maturase K (matK) for plants. The same databases were used by Norena et al. (2018).

Methods

Nucleotide data was retrieved using Entrez Direct (Kans 2013). In April 2020, we downloaded all the records from the Nucleotide database containing the query "Peru" using the following command, **<esearch -db nucleotide -query "Peru" | efetch -format gb > peru.gb**>. The output of this search, a 30 Gb file in genbank format, was used to extract information such as the journal and organism of the published sequence. In Nucleotide, information of the institution that submitted the genetic sequence is stored in the "journal" variable. A file containing only unique journal names was also created to count all Peruvian institutions in the database. Through *awk* scripts, we counted how many times the word "Peru" appeared in the variable journal, how many times a certain institution uploaded sequences to this database, and how many organisms have been sequenced. We also analyzed which organisms were the most sequenced by each institution in Peru.

BioProject data was also retrieved using Entrez Direct and the command **<esearch -db BioProject -query "Peru" | efetch -format xml | xtract -pattern DocumentSummary -element Project \ -block Organism -element OrganismName Supergroup \ -block Submission -element Name > BioProject.xml>**, and processed using *awk* scripts. Both, BOLD and PATRIC data were retrieved directly from their webpages, using "Peru" as a search query. This data was downloaded in tabular (tsv) and comma-separated (csv) formats, respectively. For both datasets, *cat* and *grep* scripts were used. *cat* concatenates texts to create a new output, and *grep -c*, counts the occurrences of a certain word query. *awk* scripts were additionally used for the BOLD dataset to group and count most sequenced taxonomic orders per institution. For the PATRIC dataset, *csvgrep* was used to count sequenced organisms per institution. All code and scripts are available in this GitHub repository: https://github.com/reymonera/mining_peru_sequence_DB. Finally, we searched in the Scopus reference database (https://www.scopus.com) for records with the term "genetic diversity" produced by at least one Peruvian institution.

Results

Our results are summarized in Figure 1 and Table 1. In addition, complete information of the records from each database described here can be found in the Supplementary Information associated with this publication (Romero & Castillo, 2020). We found 817 694 records associated with the term "Peru" in the Nucleotide database (Table 1). However, this number could be an overestimate of Peruvian sequences in this database. For instance, we found some records that corresponded to the Spanish institution Estacion Biológica de Donana, because the building where it is located is named "Pabellon del Peru". Thus, to be consistent with the previous report from Norena et al. (2018), we only focused on submissions by Peruvian institutions. Therefore, we found 14 488 sequences (1.77% of the records related to the term "Peru") submitted by 36 Peruvian institutions (Suppl. Inf. 2). 11 institutions submitted more than 100 records to this database (Fig. 1). Public institutions such as Instituto Nacional de Salud (INS), Universidad Nacional Mayor de San Marcos (UNMSM), Instituto Nacional de Investigación Agraria (INIA), and Instituto de Investigaciones de la Amazonía Peruana (IIAP), and private institutions such as Universidad Peruana Cayetano Heredia (UPCH) and Farmacéuticos Veterinarios S. A. C. (FARVET) submitted most of the records. In BioProject, we found 193 records related to the term "Peru" (Suppl. Inf. 3), 59 of them were submitted by 9 Peruvian institutions (Fig. 1). INS has most of the records, followed by UNMSM, Universidad Nacional Agraria La Molina (UNALM) and UPCH. In PATRIC, we found 2 959 records (Suppl. Inf. 4), 107 of them submitted by 8 Peruvian institutions (Fig. 1). Similarly, most of the records were submitted by UPCH, INS, and UNMSM. Finally, in BOLD, we found 23 968 public records associated to the term "Peru". From these, 8 249 were mined from Genbank so to avoid counting them twice, we discarded them in the final count. We found that 3 754 records were submitted by 11 Peruvian institutions (Suppl. Inf. 5). Most of the sequences were produced by the Centro de Ornitología y Biodiversidad (CORBIDI), UNMSM (mainly, from the Museo de Historia Natural), UNALM (mainly, from the Herbarium), the Instituto del Mar del Perú (IMARPE), and the Servicio Nacional de Sanidad Agraria del Perú (SENASA).

Records from the Nucleotide, BioProject and PATRIC databases are biased to pathogens probably reflecting the research field of groups that frequently submit DNA data and the direction of research funding. In the Nucleotide database, most records belong to *Pasteurella multocida*, and *Neisseria meningitidis,* followed by *Vibrio parahaemolyticus, Bacillus thuringiensis, Escherichia coli, Shewanella* sp*., Aeromonas veronii*, *Homo sapiens*, HIV, and *Mycobacterium tuberculosis* (Figure 2). 70.60% of the records belonged to Bacteria. 24.38%, to Eukaryota and 3.07%, to Viruses (Suppl. Inf. 2). In addition, we found no records of Archaea submitted by Peruvian institutions. Within Eukaryota, most of the

records belonged to clades Fungi and Viridiplantae and Phyla Chordata, Arthropoda, and Mollusca (Suppl. Inf. 2). In BioProject, *Salmonella enterica* subsp. enterica serovar Infantis is the most frequent record (Suppl. Inf. 3). PATRIC's most frequent records are pathogenic bacteria, namely, *Mycobacterium tuberculosis*, *Yersinia pestis*, *Shigella sonnei*, and *Staphylococcus aureus* (Suppl. Inf. 4). BOLD presents different results because it is a Eukaryotic database. BOLD's most frequent taxa are, in descending order, Chordata (Aves and Actinopterygii), Angiospermae, and Arthropoda (Insecta, and Arachnida) (Suppl. Inf. 5).

We found 266 articles published from authors affiliated to at least one Peruvian institution (Figure 3, Suppl. Inf. 6). Results were similar to the previous ones: UPCH, UNMSM, UNALM, and INS appeared in most of the results. Research groups collaborated mainly with institutions from the United States, Brazil, United Kingdom, Colombia, and France. Main subject areas in these investigations are Agricultural and Biological Sciences, (Bio)Medicine and Environmental Sciences (Suppl. Inf. 7).

Discussion

It has been two years since Norena et al. (2018) reported the first results of Latin American genetic data stored in public databases. These authors kindly shared their scripts and information with us to reproduce and compare results. In our view, open science and data must be promoted, thus, all our scripts and results can be freely accessed and downloaded from GitHub. Norena et al. reported 1.01% (~6 500) of records deposited by Peruvian institutions in NCBI Nucleotide. We showed an increase of more than two times (14 488) in records in only two years (Table 1). Although, our calculated occurrence (1.71%) did not change a lot because the permanent increase of Nucleotide total records. An independent report by Clark et al. (2016) compared Genbank information between a similar range of time (2014-2016) and found two to four-fold increases in both invertebrate and vertebrate data. In the case of Peru, INS and UNMSM have generated more data than all other institutions together (> 8 000 records). In BioProject we found 30.05% records submitted by Peruvian institutions (58 out of 193) doubling the occurrences found by Norena et al. (15.95%). In PATRIC, Norena et al. found 0.28% records submitted by Peruvian institutions while we found 3.62% records (107 out of 2959). Finally, in BOLD, we reported 3 754 records while Norena et al. found 3 438. In the latter case, the increase was smaller than in the other databases.

It is not a surprise that the same institutions are actively submitting data to different databases because they host strong research groups related to emerging and infectious diseases, biodiversity, and biotechnology. However, most of them are based in Lima, Peru's

capital. We believe that, to increase diversity and representativeness, funding efforts should be directed to more regional research groups and other highly diverse taxa, for instance, non-pathogenic bacteria, archaea, non-vertebrates and non-flowering plants.

We are aware of the limitations of our searches. We limited the search to the four databases mentioned above for consistency and reproducibility. We also limited the search to nucleotide sequences. Further information of protein sequences and structures can be found in the databases NCBI Protein and Protein Data Bank (Berman et al. 2000). Also, as we consider only Peruvian institutions, we did not mention international institutions based in Peru, for example, the Centro Internacional de la Papa (CIP) or the US Naval Medical Research Unit Six (NAMRU-6) that also produced sequences from Peruvian organisms. However, as both institutions are based in Lima, their inclusion did not change the main conclusions of our analysis (see Suppl. Inf. 2, 3, 4, 5). Furthermore, we relied on the information provided by the researchers which could result in heterogeneous data even if it comes from the same institution, for instance, in BioProject, we found records from INS, with different names such as "Peruvian National Institute of Health", "Instituto Nacional de Salud - Peru" , "Instituto Nacional de Salud" (Suppl. Inf. 3). Thus, we believe that each institution must develop an homogenous protocol for data submission.

Sequencing projects will definitely rise in the next months and years. It would not be a surprise, for instance, to see a rise of SARS-CoV2 genomes submitted by Peruvian institutions. In order to effectively track this information and keep it updated we encourage Peruvian institutions to create a unified online platform to frequently survey and organize sequencing data.

We agree with several members of the scientific community that in order to improve the quality of science: transparency, reproducibility, efficiency, and benefits to society, there is a need for open data (Molloy, 2013). Public databases play a big role in open science and reproducibility. Thus, the availability of sequencing data in this platform is crucial for scientific research and should be practiced and encouraged. In addition, we believe in the necessity of a national genome sequencing plan developed by public and private research institutions. This will increase the Peruvian genetic information in public databases, articulate efforts and promote networking from different institutions, and provide resources to manage several issues from several topics such as agriculture, public health and biodiversity conservation. Finally, efforts should be also focused on a country-wide capacity building initiative to train a new generation of Peruvian bioinformaticians that will be essential in future challenges to our biodiversity.

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TABLES

Table 1. Number of records associated to the term "Peru" and submitted by Peruvian institutions in four genetic public databases.

	This study			Norena et al. (2018)		
	Total data associated to the term "Peru"	Records submitted by Peruvian institutions	%	Total data associated to the term "Peru"	Records submitted by Peruvian institutions	%
Nucleotide	817 694	14 488	1.77	645 753	~6 500	1.01
BioProject	193	58	30.05	94	15	15.95
PATRIC	2 959	107	3.62	1 738	5	0.28
BOLD	-	3 754	-	-	3 438	-

FIGURES

Figure 1. Peruvian institutions that submitted most of the records to four public genetic databases; BioProject, BOLD, Nucleotide and PATRIC. For Nucleotide, we show institutions that submitted more than 100 records in this database.
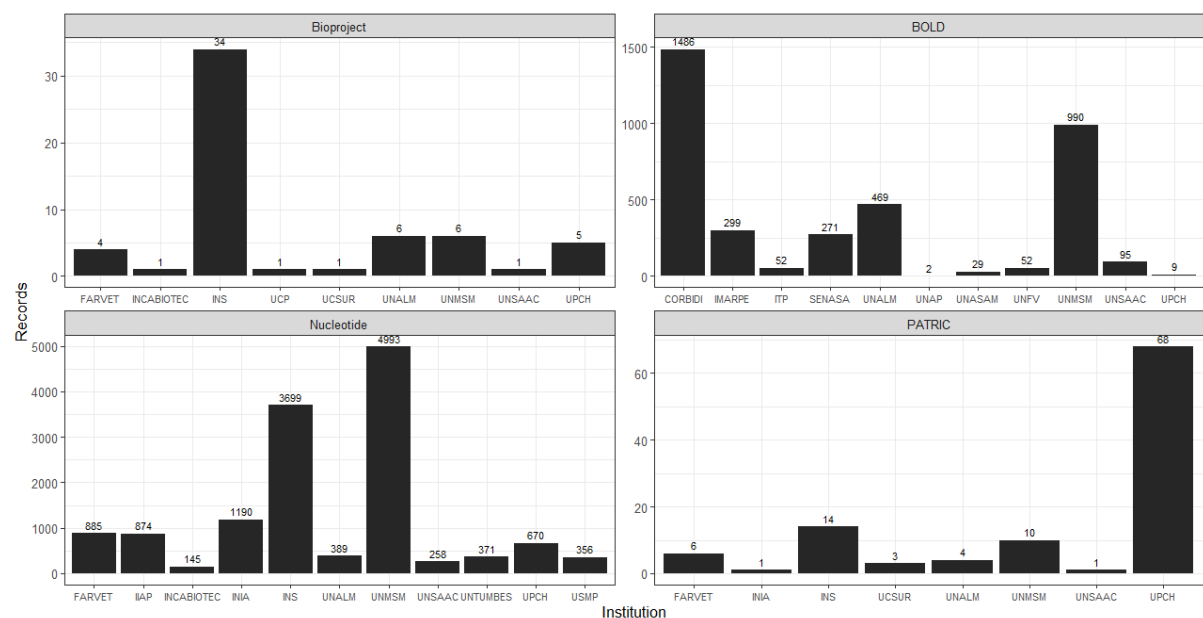
Figure 2. Most abundant records of organisms submitted by Peruvian institutions to the Nucleotide database. The figure shows organisms with more than 200 records.
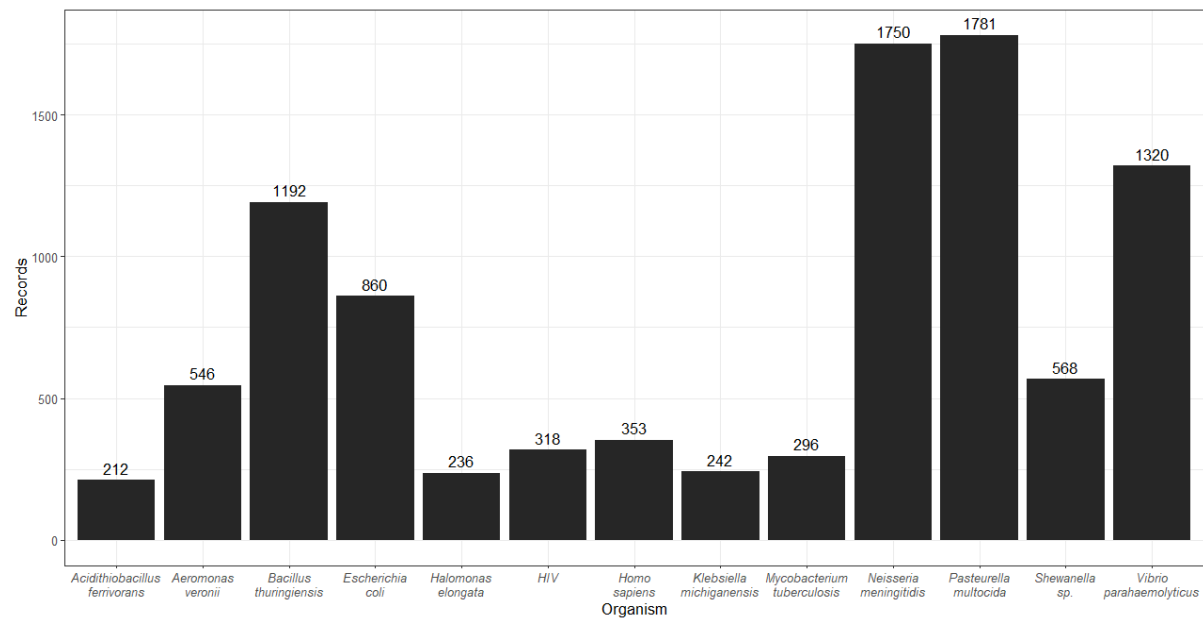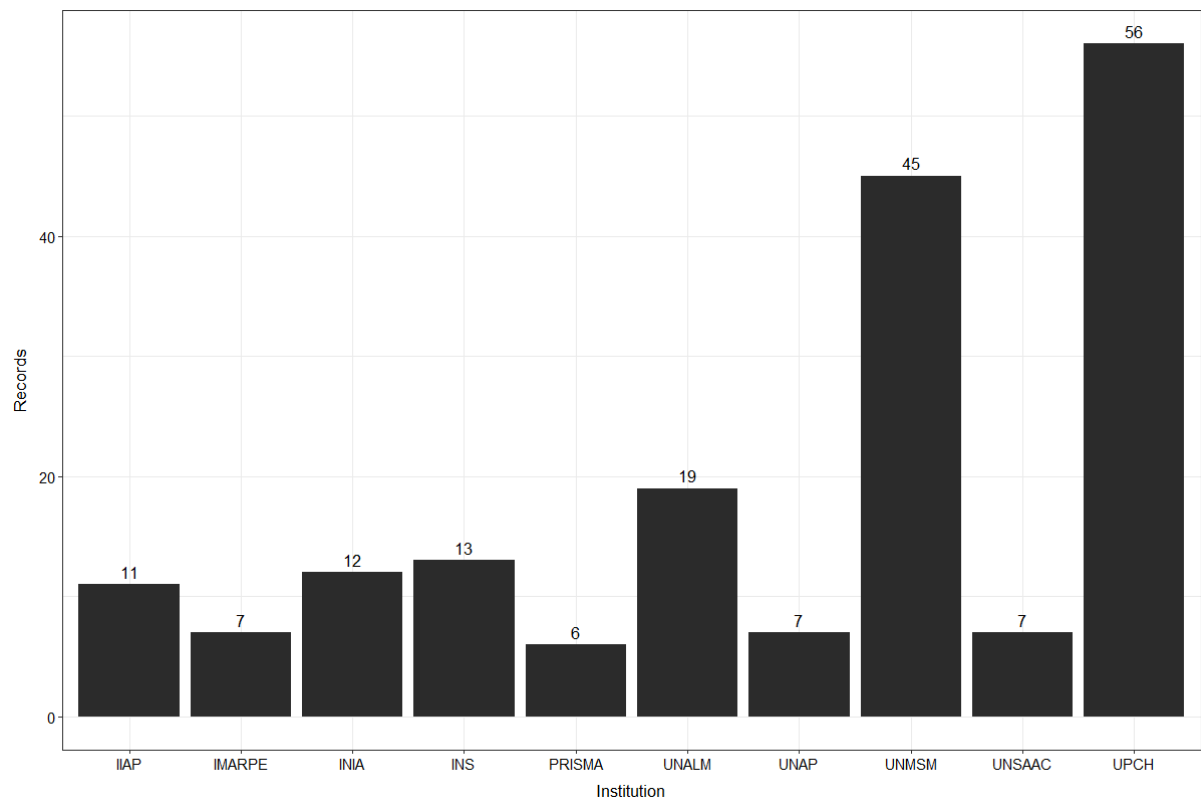
Figure 3. Peruvian institutions that appeared frequently in scientific articles about genetic diversity. The literature and affiliation search were done in Scopus.

Contribution of each author / Información sobre la contribución

PER participated in the conceptualization, data analysis, writing and review of the manuscript. CC-V participated in data analysis and writing of the manuscript

PER participó en la concepción, análisis de datos, escritura y revisión del manuscrito

CC-V participó en el análisis de los datos y la escritura del manuscrito.