

National Rural Development Programme 2014-2022

Measure 10.2 – Biodiversity

Project: TuBAvI-2 (2021-2024)

REPORT ON THE ACTIVITIES PERFORMED DURING THE THIRD AND FOURTH YEAR

UNIPD

The present report describes the activities performed from May 1st, 2023, to December 31st, 2024. The activities are described by Action.

Action 1 – Phenotypic characterization of autochthonous breeds and species

Blood samples and phenotypic characteristics were collected on animals of the following species/breeds:

- *Collo Nudo Italiana* breed (species: *Gallus gallus*) (n = 48)
- *Oca Padovana* breed (species: *Anser anser*) (n = 53)

The collection of the above information led to the completion of the activity of recording morphometric measurements and collecting biological material (blood) required by the TuBAvI-2 project. Table 1 summarizes the qualitative phenotypic characteristics (shank and skin color) of all species/breeds involved in the project, and Figure 1 depicts the descriptive statistics (mean and standard deviation) of quantitative phenotypic characteristics (body weight, body length, shank length, shank circumference, wingspan, sternum circumference) recorded on the same species/breeds. Phenotypic characteristics are presented for males and females within breed and follow the guidelines of FAO (2012)².

Action 2 – Genetic characterization of Italian breeds and species

The genetic analyses conducted by the external service/laboratory starting June 2023 enabled the acquisition of genotypes for all species/breeds involved in the project. Starting with DNA extracted from blood samples (about 2 mL) and stored in tubes with EDTA at -20°C, genotyping of animals was carried out using SNP molecular markers. Two distinct approaches were used, depending on the species:

- **Collo Nudo Italiana** and **Millefiori Piemontese breeds** (species: *Gallus gallus*) → Affymetrix Axiom Chicken 600K HD;
- **Anatra Mignon** and **Anatra Germanata Veneta** (species: *Anas platyrhynchos*), **Oca Padovana** (specie: *Anser anser*) and **Faraona Camosciata** (specie: *Numida meleagris*) **breeds** → low-coverage whole genome sequencing (WGS - coverage 4X).

The WGS approach for the species *Anas platyrhynchos*, *Anser anser* and *Numida meleagris* was used because there are no commercially available SNP chips with which to perform the same genotyping.

² FAO. (2012). Phenotypic characterization of animal genetic resources. FAO Animal Production and Health Guidelines, (11).

Action 3 - Data and information consistency check

The genotyping results were checked according to standard quality criteria. After applying filters to the genotypes, aimed at maximizing and assessing the accuracy of the results, the number of genotyped animals useful for subsequent analyses was as follows:

- *Collo Nudo Italiana* breed (species: *Gallus gallus*) (n = 47)
- *Millefiori Piemontese* breed (species: *Gallus gallus*) (n = 48)
- *Oca Padovana* breed (species: *Anser anser*) (n = 50)
- *Faraona Camosciata* breed (species: *Numida meleagris*) (n = 50)
- *Anatra Mignon* breed (species: *Anas platyrhynchos*) (n = 50)
- *Anatra Germanata* breed (species: *Anas platyrhynchos*) (n = 50)

Action 4 - Estimation of genetic and genomic indices and reproductive management in relation to new purposes

Post-quality control analyses were completed for all the species involved in the project, namely *Gallus gallus*, *Anser anser*, *Numida meleagris*, and *Anas platyrhynchos*. For individuals belonging to these species/breeds, the following genetic diversity indices were estimated: observed heterozygosity (Ho), expected heterozygosity (He), minor allele frequency (MAF), and Runs of Homozygosity (ROH), defined as DNA segments that are identical on both chromosomes within the population and their respective geographic islands (Table 2).

Action 5 - Improvement of animal genetic resources of zootechnical interest, assessment of inbreeding and genetic diversity in the considered populations (from inbreeding calculation to data modification and collection in control station under controlled environment)

To assess the improvement of genetic resources and monitor the inbreeding levels in the species *Gallus gallus*, *Anser anser*, *Numida meleagris*, and *Anas platyrhynchos*, specific indices (F_{HOM} and F_{ROH}) were estimated for each individual, based on the excess of homozygosity within the genome of the breeds. The results, presented as breed averages, are shown in Table 2. The values, ranging from 0 to 1, indicate a low level of inbreeding and a high level of genetic diversity for the two breeds of *Gallus gallus*. However, for the other species/breeds, the indices suggest a high level of inbreeding and, consequently, a low level of genetic diversity.

Action 6 - Monitoring of genetic diversity

The data from the TuBAVI-2 project for the species *Gallus gallus* were integrated with those from the previous TuBAVI project for the same species. This allowed the estimation of phylogenetic relationships using Reynolds genetic distances and provided insights into the genetic proximity among breeds within the Italian local poultry heritage (Figure 2). To further enhance the analysis of local poultry biodiversity, the entire dataset of breeds distributed across the national territory was expanded with genotypes from 167 breeds from around the world. This integration enabled the evaluation of genetic distances between Italian breeds and those from other countries on a global scale, as well as the measurement of biodiversity levels across nations. The results indicate that the Italian local poultry heritage exhibits high genetic diversity (Figure 3 and Figure 4).

Action 9 - Processing of the information collected (e.g., development of indicators and indices

to minimize the environmental impact of breeding, calculation of planned mating, etc.)

Using the genotypes obtained from the TuBAvI-2 project and the previous TuBAvI project for the species *Gallus gallus*, an investigation was conducted to identify genes involved in the pigmentation of eggshells and shank coloration (Table 3).

Additionally, with the genotypes of the animals belonging to the species *Anas platyrhynchos* (breeds: Mignon and Germanata Veneta), a genome-wide association study was performed. The Mignon and Germanata Veneta had divergent morphometric characteristics, as evaluated during the implementation of Action 1. Therefore, the genome-wide association analysis enabled the identification of candidate genes associated with the distinct developmental traits of the two breeds (Table 4).

Action 10 – Information actions, dissemination and preparation of thematic technical reports and technical-scientific reports

The following activities were carried out under this action:

- a) participation in updating the project website (<https://www.pollitaliani.it/>);
- b) participation in preparing new dissemination material (e.g. posters with pictures of cockerels of the breeds in conservation) for events like exhibition events in which the consultant (ANCI) participated;
- c) updating the data sheets of Veneto poultry species/breeds:
 - Species: *Meleagris gallopavo*. Breeds: Bronzato Comune, Ermellinato di Rovigo.
 - Species: *Gallus gallus*. Breeds: Millefiori di Lonigo, Ermellinata di Rovigo, Pepoi, Padovana (Dorata, Camosciata and Argentata), Polverara (Bianca and Nera), Robusta Maculata, Robusta Lionata;
- d) participation to the final TuBAvI-2 project meeting held in Lodi on December 6, 2024, with the presentation titled: "Analysis of genetic variability in local poultry resources";
- e) participation in national and international congresses with oral/poster presentation:
 - Cendron, F., Cassandro, M., Penasa, M. (2023). Copy number variants in 23 Italian local chicken breeds. In: Book of Abstracts of the 25th National Congress of the Animal Science and Production Association (ASPA), 13-16 June, Monopoli (BA), Italy. ITALIAN JOURNAL OF ANIMAL SCIENCE, 22(Suppl. 1):99-100. (Abstr. O481). (Oral Presentation).
 - Cendron, F., Penasa, M., Cassandro, M. (2023). Genome-wide detection and analysis of copy number variation in Italian indigenous chicken breeds. In: Proceedings of the 12th European Symposium on Poultry Genetics, 8-10 November, Hannover, Germany, p. 46. (Poster).
 - Perini, F., Cendron, F., Lasagna, E., Cassandro, M., Penasa, M. (2024). Exploring the genomic basis of shank and eggshell coloration in Italian native chicken breeds. In: Book of Abstracts of the 75th Annual Meeting of the European Association of Animal Science, September 1–5, Florence, Italy, vol. 34:797. (Poster).
 - Cendron, F., Perini, F., Lasagna, E., Soglia, D., Schiavone, A., Cassandro, M., Penasa, M. (2024). Genetic characterization of Collo Nudo Italiana and Millefiori Piemontese chicken breeds through high-density SNP genotyping. In: Book of Abstracts of the 75th Annual Meeting of the European Association of Animal Science, September 1–5, Florence, Italy, vol. 34:475. (Poster).
- f) publication of the following scientific papers:

- Cendron, F., Cassandro, M., Penasa, M. (2024). Genome-wide investigation to assess copy number variants in the Italian local chicken population. *JOURNAL OF ANIMAL SCIENCE AND BIOTECHNOLOGY*, 15:2.
- Perini, F., Cendron, F., Lasagna, E., Cassandro, M., Penasa, M. (2024). Genomic insights into shank and eggshell color in Italian local chickens. *POULTRY SCIENCE*, 103:103677.
- Di Iorio, M., Marelli, S. P., Antenucci, E., Madeddu, M., Zaniboni, L., Belcredito, S., Rusco, G., Schiavone, A., Soglia, D., Penasa, M., Castellini, C., Buccioni, A., Marzoni, M., Maiuro, L., Iaffaldano, N., Cerolini, S. (2024). A comparative study on semen quality and cryopreservation ability in Italian native chicken breeds. *ITALIAN JOURNAL OF ANIMAL SCIENCE*, 23:1704-1718.
- Perini, F., Cendron, F., Castellini, C., Iaffaldano, N., Marzoni, M., Buccioni, A., Soglia, D., Schiavone, A., Cerolini, S., Lasagna, E., Cassandro, M., Penasa, M. (2025). Genome-wide analysis of Collo Nudo Italiana and Millefiori Piemontese local chicken breeds: genetic variability and structure analysis in the context of Italian chicken biodiversity. *ITALIAN JOURNAL OF ANIMAL SCIENCE*, 24:137-148.
- Cappone, E. E., Zambotto, V., Mota-Gutierrez, J., Soglia, D., Daniele, G. M., Cianciabella, M., Pieroni, A., Soukand, R., Penasa, M., Buccioni, A., Marzoni, M., Iaffaldano, N., Castellini, C., Cerolini, S., Forte, C., Schiavone, A. (2025). Native Italian poultry products: the factors influencing consumer perceptions. *ITALIAN JOURNAL OF ANIMAL SCIENCE*, 24:347-360.

FIGURES AND TABLES

Table 1 – Shank and skin color of sampled animals.

Breed	Species	Sex	N° animals	Shank color	Skin color
Collo Nudo Italiana	<i>Gallus gallus</i>	Male	21	Yellow	White
		Female	26	Yellow	White
Faraona Camosciata	<i>Numida meleagris</i>	Male	25	Dark orange	Grey
		Female	26	Brown	Grey
Anatra Mignon	<i>Anas platyrhynchos</i>	Male	12	Orange	White
		Female	43	Orange	White
Anatra Germanata Veneta	<i>Anas platyrhynchos</i>	Male	14	Orange	White
		Female	41	Orange	White
Oca Padovana	<i>Anser anser</i>	Male	23	Orange	White/Pink
		Female	39	Orange	White/Pink

Table 2 – Indices of genetic diversity and coefficients of inbreeding.

Breed	N° animals	MAF		He		Ho		F _{HOM}		F _{ROH}	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Collo Nudo Italiana	47	0.311	0.170	0.484	0.107	0.469	0.128	-0.001	0.066	0.003	0.004
Millefiori Piemontese	48	0.245	0.201	0.383	0.112	0.428	0.149	0.149	0.405	0.016	0.009
Oca Padovana	50	0.199	0.069	0.129	0.071	0.143	0.149	0.473	0.174	0.451	0.376
Faraona Camosciata	50	0.209	0.159	0.219	0.145	0.221	0.280	0.528	0.098	0.536	0.138
Anatra Mignon	50	0.191	0.132	0.238	0.146	0.244	0.171	0.368	0.162	0.313	0.125
Anatra Germanata Veneta	50	0.192	0.127	0.268	0.147	0.271	0.261	0.304	0.183	0.372	0.222

MAF = minimum allele frequency; He = expected heterozygosity; Ho = observed heterozygosity; F_{HOM} = coefficient of inbreeding based on homozygosity regions; F_{ROH} = coefficient of inbreeding based on runs of homozygosity. SD = standard deviation.

Table 3 – Genes responsible for eggshell and shank color.

Shank		Eggshell	
Chromosome and position	Genes	Chromosome and position	Genes
1:100867039-101831504	<i>CHODL</i> , <i>TMPRSS15</i> , <i>NCAM2</i>	2:61184687-61271239	<i>JARID2</i>
2:38800153-38800475	<i>EOMES</i> , <i>CMC1</i> , <i>AZI2</i> , <i>RBMS3</i>	4:26751167-30211214	<i>PCDH18</i>
Z:11112490-12254700	<i>SLC1A3</i> , <i>RANBP3L</i> , <i>SLC45A2</i>	5:15963249-15987149	<i>PNPLA2</i> , <i>SLC25A22</i>
Z:18925613-18969905	<i>ERCC8</i>	12:15839118-16014277	<i>MITF</i> , <i>FAM19A4</i> , <i>ARL6IP5</i> , <i>UBA3</i>
Z:23446708-23450910	<i>F2RL1</i>	Z :10028822-10307280	<i>NPR3</i> , <i>TARS</i> , <i>ADAMTS12</i> , <i>SLC45A2</i>
Z:31376546-32699330	<i>NFIB</i> , <i>ZDHC21</i> , <i>CER1</i> , <i>PSIP1</i> , <i>BNC2</i> , <i>TYRP1</i>		
Z:78846780-79172113	<i>CDKN2A</i> , <i>CDKN2B</i>		
Z:78846780-79172113	<i>MTAP</i> , <i>FEM1C</i>		
Z:78846780-79213873	<i>TRIM36</i> , <i>GRAMD3</i>		

Table 4 – Candidate genes responsible for the divergent phenotype between duck breeds.

Chromosome	Start	End	Gene	Chromosome	Start	End	Gene
1	25709525	25714432	<i>ANKRD7</i>	13	8797007	8815208	<i>AKAP8</i>
1	189778155	189959445	<i>DDX10</i>	13	8768502	8774393	<i>GIPC1</i>
1	62138472	62175230	<i>DNM1L</i>	13	15769158	15778839	<i>GRM2</i>
1	62191937	62304900	<i>FGD4</i>	13	15611356	15665218	<i>POC1A</i>
1	123404775	123554825	<i>KDM6A</i>	13	15765581	15769004	<i>RRP9</i>
1	25722095	25727656	<i>LSM8</i>	13	15780564	15800993	<i>TEX264</i>
1	206209032	206225355	<i>NUMA1</i>	14	1103229	1168940	<i>NR3C1</i>
1	206231025	206239198	<i>RNF121</i>	20	10912905	10918873	<i>CCDC183</i>
2	571306	575439	<i>CCDC166</i>	20	10906954	10912685	<i>SBD5</i>
2	119921792	119972492	<i>COL6A6</i>	20	75662	99295	<i>SMTNL2</i>
2	543279	568682	<i>MAPK15</i>	23	18092	27667	<i>ADD2</i>
2	119861581	119889026	<i>PIK3R4</i>	23	27824	29657	<i>FIGL4</i>
3	13228755	13264072	<i>ABHD12</i>	23	62049	81621	<i>GMCL1</i>
3	25757737	25782715	<i>EDARADD</i>	23	39860	61357	<i>HK2</i>
3	25680314	25713321	<i>ERO1B</i>	23	15109	17877	<i>SNRNP27</i>
3	119252132	119259773	<i>GAREM2</i>	27	1913045	1915906	<i>ARMC12</i>
3	25653454	25678357	<i>GPR137B</i>	27	846183	867861	<i>CELSR2</i>
3	119227500	119251352	<i>HADHA</i>	27	1916083	1917086	<i>CLPS</i>
3	119212635	119227318	<i>HADHB</i>	27	829667	839920	<i>CPNE5</i>
3	118846356	118854857	<i>KIFC1</i>	27	875963	884044	<i>ELAPOR1</i>
3	41862733	41871547	<i>MAP10</i>	27	1882276	1905786	<i>FKBP5</i>
3	119122049	119142500	<i>SELENOI</i>	27	1918895	1922650	<i>LHFPL5</i>
3	119098256	119098328	<i>TRNAK-CUU_4</i>	27	908510	924318	<i>LMOD1</i>
3	119106687	119106759	<i>TRNAK-CUU_5</i>	27	840565	842280	<i>PPIL1</i>
3	119113384	119113456	<i>TRNAK-CUU_6</i>	27	842399	845079	<i>PSRC1</i>
4	6291006	6327110	<i>TACR3</i>	27	869216	875281	<i>SARS1</i>
5	65317872	65329359	<i>EIF4G2</i>	27	901223	904135	<i>SHISA4</i>
5	29946634	30018899	<i>PTPRJ</i>	27	1837777	1842374	<i>SMPD2</i>
6	35303317	35325685	<i>MKI67</i>	27	1923359	1949477	<i>SRPK1</i>
6	35198786	35292964	<i>PTPRE</i>	27	1843163	1861686	<i>TEAD3</i>
7	28464795	28481051	<i>COL18A1</i>	27	929213	938128	<i>TIMM17A</i>
7	28414738	28459898	<i>PCBP3</i>	27	888178	890731	<i>TMEM167B</i>
7	28459954	28464995	<i>SLC19A1</i>	27	1871600	1880611	<i>TULP1</i>
8	27692565	28133935	<i>DAB1</i>	28	3813525	3820305	<i>EFCAB3</i>
8	28213322	28235065	<i>OML1</i>	28	3826217	3845163	<i>ITGB3</i>
10	44027	48988	<i>PSMD10</i>	28	3848260	3849639	<i>RPRML</i>
10	49946	77855	<i>VSIG1</i>	29	5697591	5722473	<i>ATCAY</i>

Figure 1 – Mean and standard deviation of quantitative phenotypic characteristics by breed and sex of the animals.

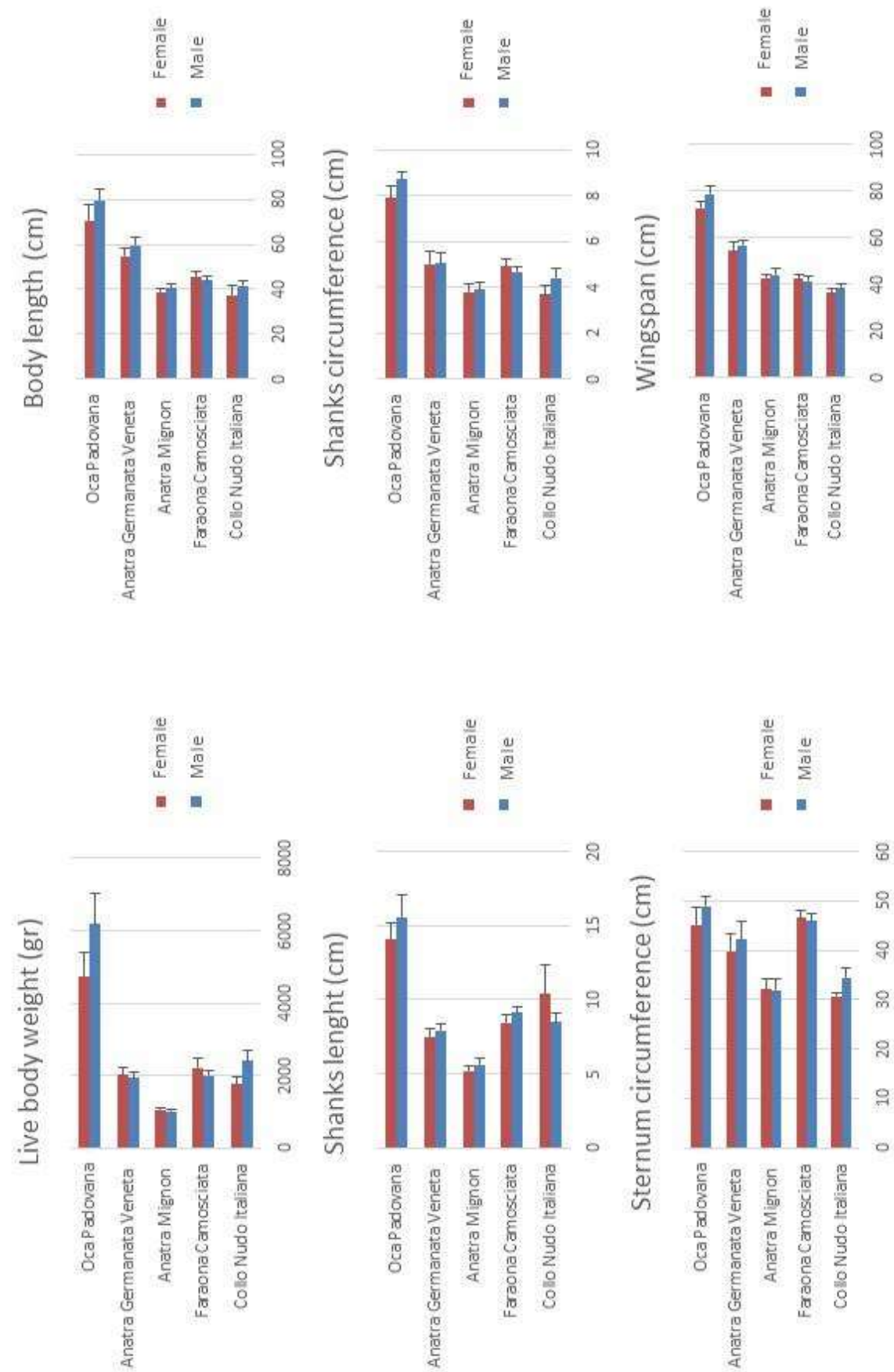
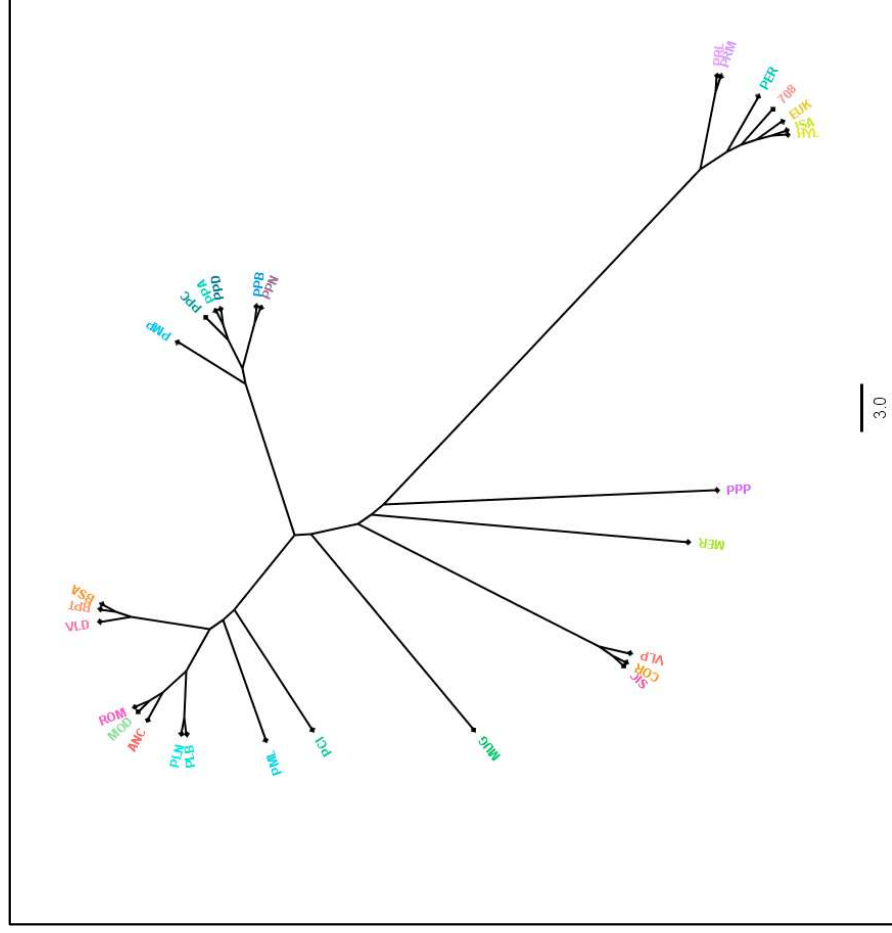


Figure 2 – Phylogenetic tree for chicken breeds (species: *Gallus gallus*) from the TuBAv1 and TuBAv1-2 projects, obtained using Reynolds genetic distances.



Ancona (ANC), Bianca di Saluzzo (BSA), Bionda Piemontese (BPT), Cornuta di Caltanissetta (COR), Collo Nudo Italiana (PCI), Livorno Bianca (PLB), Livorno Nera (PLN), Millefiori Piemontese (PMP), Mericanella della Brianza (MER), Modenese (MOD), Mugellese (MUG), Ermellinata di Rovigo (PER), Millefiori di Lonigo (PML), Padovana Argentata (PPA), Polverara Bianca (PPB), Padovana Camosciata (PPC), Padovana Dorata (PPD), Polverara Nera (PPN), Pepoi (PPP), Robusta Lionata (PRL), Robusta Maculata (PRM), Romagnola (ROM), Siciliana (SIC), Valdarnese (VLD), Valpatani (VLP), Broiler Ross 708 (708), Eureka (EUK), Hy-line (HYL), ISA Brown (ISA).

Figure 3 – Genetic background of Italian breeds within the global poultry context.

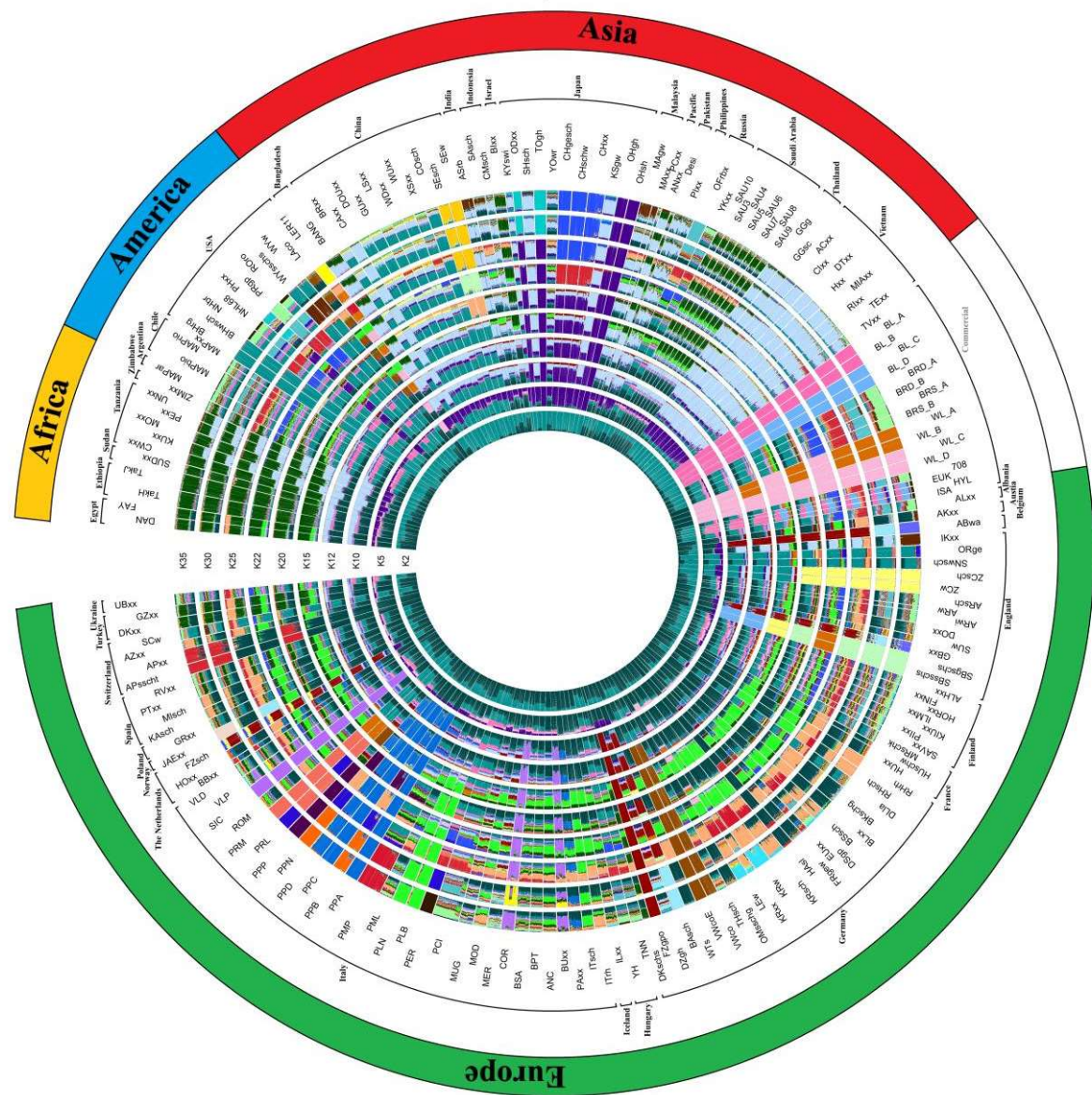


Figure 4 – (A) Expected heterozygosity, (B) Observed heterozygosity, (C) Inbreeding coefficient due to excess of homozygosity, and (D) Estimated homozygosity coefficient based on Runs of Homozygosity for each chicken breed group within the country of origin.

