Sèyi Fridaïus Ulrich Sédric Vanvanhossou

Genetic Evidences of Adaptive and Productive Features in the West African Indigenous Cattle Population in Benin

A dissertation submitted to the Faculty of
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Environmental Management, Justus Liebig University Gießen,
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Submitted by

Sèyi Fridaïus Ulrich Sédric Vanvanhossou

Born in Agouna, Benin

Gießen, 2022

With the consent of the Faculty of Agricultural Science, Nutritional Science, and Environmental Management, Justus Liebig University Gießen

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To my family, for all the love and sacrifice.

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Summary

The lack of proper genetic characterization of the West African cattle genetic resources is a major limitation for their sustainable management and the improvement of cattle production in this region. The current study examines the genetic background of adaptive and productive features in the indigenous cattle breeds kept in Benin. For this purpose, different approaches, including up-to-date genomic methodologies such as selection signature analysis, genome-wide association study (GWAS), and next generation sequencing were applied to genetically characterize the Beninese indigenous taurines (Lagune, Somba) and crossbreeds (Borgou, Pabli), as representatives of West African cattle genetic resources.

An overview of the production systems and current institutional management of cattle breeds in Benin summarizes in Publication 1 the existing challenges to promote animal genetic resources and to sustain cattle production. Next, strategies for genetic evaluation and breed selection were explored with respect to the breeding goals and the traits (e.g., robustness) valued by smallholders, for efficient and durable improvements of farmer management practices and animal performances.

In Publication 2, genotype data were generated with medium-density single nucleotide polymorphism (SNP) panels from current Dahomey cattle samples (30 animals) and current Beninese indigenous cattle samples (449 animals from four breeds: Lagune, Somba, Borgou and Pabli). These genotype data were contrasted with available historical genotypes from Beninese, other West African, Asian and European cattle, to test for admixture, to compute admixture proportions and genomic inbreeding, and to identify selection signatures. The findings substantiated the high genetic proximity of Dahomey cattle to the Lagune, in contrast to the European taurine. However, high inbreeding rate in the Dahomey cattle population suggested imperative strategies (including exchange of breeding stock) to increase diversity in the breed. Moreover, evidence of admixture with Zebu cattle was observed in the local cattle breeds sampled in Benin, especially the Borgou. The latter breed displayed an important reduction of African taurine (AFT) ancestral proportion, in comparison with historical data. Nevertheless, the most recent selection signatures identified in the investigated breeds were associated with economic traits (reproduction, growth, milk) and adaptive traits (immunity, feed efficiency), irrespective of the admixture proportions. More interestingly, specific genomic footprints (rarely reported in other African breeds), and hosting genes related to disease resistance (e.g., DSB, BOLA-DYA) were detected in the Somba.

Furthermore, multi-breed genetic parameter estimations and GWAS were performed for six morphometric measurements (height at withers, HAW, sacrum height, SH, heart girth, HG, hip

width, HW, body length, BL and ear length, EL), considering the genotype and the fixed effects (age, sex, agro-ecological zones) of the 449 current Beninese indigenous cattle samples (Publication 3). The estimated SNP-based heritabilities for the morphometric traits ranged between 0.46 ± 0.14 (HG) and 0.74 ± 0.13 (HW). Phenotypic and genetic correlations ranged from 0.25 ± 0.05 (HW-BL) to 0.89 ± 0.01 (HAW-SH), and from 0.14 ± 0.10 (HW-BL) to 0.85 ± 0.02 (HAW-SH), respectively. A total of 28 SNP showed significant associations with the investigated traits. The SNP were located in close proximity (± 25kb) to 15 genes related to height or body size (e.g. *PIK3R6*, *PIK3R1*), conformation, growth or carcass traits (*VEPH1*, *CNTNAP5*, *GYPC*), and stress or immune response (*PTAFR*, *PBRM1*, *ADAMTS12*).

Six milk protein genes *CSN1S1*, *CSN2*, *CSN1S2*, *CSN3*, *LALBA*, and *LGB* were targeted in Publication 4 to study their polymorphisms, and casein haplotypes in Beninese indigenous cattle. Next generation sequencing of the genes' exons, flanking intron regions and parts of the 5′-upstream regions, applied on 67 Beninese indigenous cattle, revealed a total of 1058 genetic variants with 731 previously undescribed. Within the identified genetic variants, 12 polymorphisms determined 17 milk protein variants, including two for *CSN1S1* (B, C), three for *CSN2* (A1, A2, L), two for *CSN1S2* (A, B), three for *CSN3* (A, B, K), four for *LALBA* (A, B, E, F), and three for *LGB* (B, B1, K). In this regard, the characterization of four novel milk protein variants (*CSN3*^{K}, *LALBA*^{F}, *LGB*^{B1} and *LGB*^{K}*)* in the Beninese cattle breeds confirms their rich and unique genetic diversity. Additionally, five main haplotypes were constructed based on variants within the casein genes (*CSN1S1-CSN2-CSN1S2-CSN3)*, but the haplotypes B-A1-A-B, B-A1-A-A and C-A2-A-B were the most frequent.

Finally, Publication 5 reports the agricultural and ecological utilization or importance of the Dahomey cattle in European countries. The breed corresponds to the Beninese Lagune cattle breed, which was introduced to Europe in the early 1900's. A web-based open-closed questionnaire was used to investigate the motivations and management practices of farmers keeping the Dahomey cattle in Germany, Switzerland and Austria. The results revealed increasing interests in the Dahomey cattle, which were mainly kept in small herds (seven animals on average) for landscape maintenance and meat production or breeding stock. Farmers appreciated various features (e.g., resistance to disease, calving ease, low feeding requirement) in the Dahomey cattle, and were dedicated to the promotion of the breed.

Overall, this study evidenced a high genetic correlation between adaptive and productive features in the West African indigenous cattle in Benin. The current findings corroborate previous knowledge on West African or other African indigenous cattle. Their implications for the sustainable management of the breeds are extensively discussed. Further investigations to

consolidate these results and improve the management of West African cattle genetic resources are also suggested.

Zusammenfassung

Die fehlende genetische Charakterisierung der genetischen Ressourcen westafrikanischer Rinder behindert deren nachhaltige Nutzung und die Verbesserung der Rinderproduktion in der Region erheblich. In der vorliegenden Studie wird der genetische Hintergrund der adaptiven und produktiven Merkmale westafrikanischer indigener Rinderrassen im Benin untersucht. Zu diesem Zweck wurden verschiedene Ansätze, einschließlich der aktuellen genomischen Methoden wie Selektionssignaturanalyse, genomweite Assoziationsstudie (GWAS) und Next-Generation-Sequenzierung angewandt, um die einheimischen Taurinrassen (Lagune, Somba) und Kreuzungsrassen (Borgou, Pabli) in Benin als typische westafrikanische Rinderrassen genetisch zu charakterisieren.

Ein Überblick über die Produktionssysteme und das vorhandene institutionelle Management von Rinderrassen im Benin fasst in Publikation 1 die bestehenden Herausforderungen bei der Förderung von tiergenetischen Ressourcen und der nachhaltigen Rinderproduktion zusammen. Daher wurden Strategien für die genetische Bewertung und die Zuchtselektion im Hinblick auf die Zuchtziele und die von den Kleinbauern geschätzten Merkmale (z.B. Robustheit) evaluiert, um die Managementpraktiken der Landwirte und die Leistungen der Tiere effizient und dauerhaft zu verbessern.

In Publikation 2 wurden eigene Genotypdaten mit einer mittleren Anzahl von Einzelnukleotid-Polymorphismen (SNP) von aktuellen Proben von Dahomey-Rindern (30 Tiere) und lokalen Rindern aus dem Benin (449 Tiere aus Lagune, Somba, Borgou und Pabli) generiert. Diese Genotypdaten wurden mit den historischen Genotypen von beninischen, anderen westafrikanischen, asiatischen und europäischen Rindern verglichen, wobei formale Tests auf Beimischung (Admixture) von Rasseanteilen, Berechnungen von Admixture-Anteilen und genomischer Inzucht sowie Selektionssignaturen angewandt wurden. Die Ergebnisse bestätigen die große genetische Verwandtschaft des Dahomey-Rindes mit dem Lagune-Rind, im Gegensatz zu europäischen Rinderrassen. Die hohe Inzuchtrate in der Dahomey-Rinderpopulation erfordert jedoch dringend Strategien (einschließlich des Austauschs von Zuchttieren), um die Vielfalt der Rasse zu erhöhen. Darüber hinaus wurden bei den im Benin untersuchten lokalen Rinderrassen, insbesondere bei den Borgou, Anzeichen für Kreuzungen mit Zeburassen festgestellt. Die Borgou-Rasse zeigte im Vergleich zu historischen Daten eine deutliche Abnahme der ursprünglichen afrikanischen Taurin (AFT)-Anteile. Die identifizierten Selektionssignaturen standen jedoch, unabhängig von den Beimischungsverhältnissen in den untersuchten Rassen, mit wirtschaftlichen Merkmalen (Reproduktion, Wachstum, Milch) und adaptiven Merkmalen (Immunität, Futtereffizienz) in Zusammenhang. Interessanterweise

wurden bei den Somba spezifische genomische Merkmale (über die bei anderen afrikanischen Rassen selten berichtet wurde) festgestellt, die Krankheitsresistenz-relevante Gene (z. B. *DSB, BOLA-DYA*) beherbergen.

Außerdem wurden für sechs morphometrische Merkmale (Widerristhöhe (HAW), Kreuzbeinhöhe (SH), Brustumfang (HG), Hüftbreite (HW), Körperlänge (BL) und Ohrlänge (EL)) anhand des Genotyps und der fixen Effekte (Alter, Geschlecht, agro-ökologische Zonen) der 449 aktuellen beninischen einheimischen Rinderproben genetische Parameterschätzungen und GWAS durchgeführt (Publikation 3). Die geschätzten SNP-basierten Heritabilitäten für die morphometrischen Merkmale lag zwischen 0,46 ± 0,14 (HG) und 0,74 ± 0,13 (HW). Phänotypische und genetische Korrelationen reichten von 0,25 ± 0,05 (HW-BL) bis 0,89 ± 0,01 (HAW-SH) bzw. von 0,14 ± 0,10 (HW-BL) bis 0,85 ± 0,02 (HAW-SH). Insgesamt 28 SNP zeigten einen signifikanten Zusammenhang mit den untersuchten Merkmalen. Die SNP befanden sich in direkter Nähe (± 25kb) zu 15 Genen, die mit der Körpergröße (z. B. *PIK3R6, PIK3R1*), dem Körperbau, dem Wachstum oder den Schlachtkörpereigenschaften (*VEPH1, CNTNAP5, GYPC*) sowie der Stress- oder Immunreaktion (*PTAFR, PBRM1, ADAMTS12*) zusammenhängen.

Publikation 4 fokussierte auf die sechs Milchproteingene *CSN1S1, CSN2, CSN1S2, CSN3, LALBA* und *LGB*, um deren Polymorphismen und Kasein-Haplotypen bei einheimischen Rindern aus dem Benin zu untersuchen. Die Next-Generation-Sequenzierung der Exons, der flankierenden Intron-Regionen und von Teilen der 5′-Upstream-Regionen der Gene bei 67 einheimischen Rindern aus dem Benin ergab insgesamt 1058 genetische Varianten, von denen 731 bisher unbeschrieben waren. Innerhalb der identifizierten genetischen Varianten teilten sich 12 Polymorphismen in 17 Milchproteinvarianten auf, darunter zwei für *CSN1S1* (B, C), drei für *CSN2* (A1, A2, L), zwei für *CSN1S2* (A, B), drei für *CSN3* (A, B, K), vier für *LALBA* (A, B, E, F) und drei für *LGB* (B, B1, K). In diesem Zusammenhang beweist die Charakterisierung von vier neuen Milchproteinvarianten ($CSN3^{K}$, $LALBA^{F}$, LGB^{B1} und LGB^{K}) in den beninischen Rinderrassen deren reiche und einzigartige genetische Vielfalt. Zudem wurden fünf Haupt-*CSN1S1-CSN2-CSN1S2-CSN3*-Haplotypen konstruiert, wobei die Haplotypen B-A1-A-B, B-A1-A-A und C-A2-A-B am häufigsten waren.

Schließlich berichtet Publikation 5 über die landwirtschaftliche und ökologische Nutzung bzw. den Wert des Dahomey-Rindes in europäischen Ländern. Die Rasse entspricht den beninischen Lagune-Rindern, die seit Anfang der 1900er Jahre in Europa eingeführt wurden. Die Motivation und die Managementpraktiken der Landwirte, die Dahomey-Rinder in Deutschland, der Schweiz und Österreich halten, wurden anhand offener Fragen in einem Online-Fragebogen untersucht. Die Ergebnisse zeigen ein zunehmendes Interesse an den Dahomey-Rindern, die

hauptsächlich in kleinen Herden (durchschnittlich sieben Tiere) zur Landschaftspflege und Fleischproduktion oder als Zuchttiere gehalten werden. Die Landwirte schätzten verschiedene Merkmale des Dahomey-Rindes (z. B. Krankheitsresistenz, Leichtkalbigkeit, geringer Futterbedarf) und engagierten sich für die Förderung dieser Rasse.

Insgesamt wurde in dieser Studie eine hohe genetische Korrelation zwischen adaptiven und produktiven Merkmalen bei den westafrikanischen einheimischen Rindern im Benin nachgewiesen. Die aktuellen Ergebnisse bestätigen frühere Erkenntnisse über westafrikanische oder andere in Afrika einheimische Rinder. Ihre Bedeutung für die nachhaltige Haltung der Rassen wird ausführlich diskutiert. Weitere Untersuchungen zur Vertiefung dieser Ergebnisse und zur Verbesserung des Managements der genetischen Ressourcen westafrikanischer Rinder werden ebenfalls vorgeschlagen.

Résumé

La faible caractérisation génétique des races bovines d'Afrique de l'Ouest constitue une entrave majeure à leur gestion durable et à l'amélioration de la production bovine dans la région. La présente étude a examiné les facteurs génétiques déterminant les aptitudes adaptatives et productives des races bovines indigènes du Bénin. À cette fin, différentes approches méthodologiques, y compris des méthodes génomiques modernes telles que la détection de signatures de sélection, l'étude d'association pangénomique (GWAS) et le séquençage de nouvelle génération, ont été appliquées pour caractériser sur le plan génétique les taurins indigènes (Lagunaire, Somba) et les métis (Borgou, Pabli) du Benin, en tant que représentants des ressources génétiques bovines ouest-africaines.

Un état des lieux des systèmes de production et de la gestion institutionnelle actuelle des races bovines au Bénin a été réalisé dans le premier chapitre (publication 1) et a fait ressortir les défis à relever pour la promotion des ressources génétiques animales et le développement durable de la production bovine. Ensuite, les stratégies d'évaluation génétique et de sélection des races ont été identifiées et suggérées en mettant l'accent sur les objectifs de sélection et les traits (tels que la robustesse des animaux) privilégiés par les petits exploitants, afin d'assurer une amélioration efficace et durable des pratiques de gestion des agriculteurs et des performances des animaux.

Dans la publication 2, les données génotypiques de récents échantillons de bovins Dahomey (30 animaux sélectionnés en Europe) et de quatre races locales du Benin (au total 449 bovins de races Lagunaire, Somba, Borgou et Pabli sélectionnés au Bénin) ont été générées avec des panels de polymorphisme nucléotidique (SNP) de densité moyenne. Les données génotypiques nouvellement générées ont été comparées aux génotypes historiques préexistants des bovins béninois, ainsi que ceux d'autres bovins d'Afrique de l'Ouest, d'Asie et d'Europe afin de tester d'éventuels métissages, de calculer les proportions de métissages et de consanguinité génomique, et d'identifier les signatures de sélection. Les résultats ont confirmé la grande proximité génétique des bovins Dahomey (sélectionnés en Europe) avec les bovins Lagunaires, contrairement aux races taurines européennes. Le taux de consanguinité élevé dans la population bovine du Dahomey suggère des stratégies impératives (y compris l'échange de reproducteurs) pour augmenter la diversité de la race. Par ailleurs, des indices de métissage avec des bovins zébus ont été observés au niveau des races bovines locales échantillonnées au Bénin, notamment le Borgou. Cette dernière race présentait une réduction importante de la proportion ancestrale des taurins africains, comparativement aux données historiques. Cependant, les signatures de sélection récentes identifiées dans les races étudiées étaient associées à des traits économiques (reproduction, croissance, lait) et adaptatifs (immunité,

efficacité alimentaire), indépendamment des proportions de métissage. Encore plus intéressant, des empreintes génétiques spécifiques (rarement rapportées dans d'autres races africaines), et abritant des gènes liés à la résistance aux maladies (ex., DSB, BOLA-DYA) ont été détectées chez la race bovine Somba.

En outre, l'estimation des paramètres génétiques ainsi que l'analyse GWAS multi-races ont été effectuées pour étudier l'architecture génétique de six mesures morphométriques (hauteur au garrot, HAW ; hauteur du sacrum, SH ; périmètre cardiaque, HG ; largeur des hanches, HW ; longueur du corps, BL et longueur des oreilles, EL) chez les races bovines béninoises. Les données génotypiques des 449 animaux échantillonnés ainsi que des facteurs fixes (tels que l'âge, le sexe, zones agro-écologiques) ont été considérés à cet effet. Les héritabilités des traits morphométriques variaient entre 0,46 ± 0,14 (HG) et 0,74 ± 0,13 (HW). Les corrélations phénotypiques et génétiques entre traits morphométriques allaient de 0,25 ± 0,05 (HW-BL) à 0,89 ± 0,01 (HAW-SH), et de 0,14 ± 0,10 (HW-BL) à 0,85 ± 0,02 (HAW-SH), respectivement. Au total, 28 SNP présentaient des associations significatives avec les traits étudiés. Ces SNP sont positionnés à proximité (± 25kb) de 15 gènes impliqués dans la taille ou la conformation (ex., *PIK3R6, PIK3R1*), la croissance ou la carcasse (ex., *VEPH1, CNTNAP5, GYPC*), et dans la réponse immunitaire ou la réponse au stress (ex., *PTAFR, PBRM1, ADAMTS12*).

Dans la publication 4, six gènes de protéines du lait (*CSN1S1, CSN2, CSN1S2, CSN3, LALBA* et *LGB*) ont été ciblés pour examiner leurs polymorphismes et les haplotypes de caséine chez les races bovines indigènes du Bénin. Le séquençage de nouvelle génération des exons, introns adjacents et de certaines régions 5′ des gènes, réalisé sur 67 bovins indigènes béninois, a révélé un total de 1058 variants génétiques (SNP, InDel), dont 731 n'avaient encore jamais été décrits chez les bovins. Parmi ces variants génétiques identifiés, 12 polymorphismes induisent 17 variants de protéines du lait, dont deux pour *CSN1S1* (B, C), trois pour *CSN2* (A1, A2, L), deux pour *CSN1S2* (A, B), trois pour *CSN3* (A, B, K), quatre pour *LALBA* (A, B, E, F) et trois pour *LGB* (B, B1, K). À cet égard, l'identification de quatre nouveaux variants de protéines de lait ($CSN3^{K}$, $LALBA^{F}$, LGB^{B1} et LGB^{K}) dans les races bovines béninoises confirme leur diversité génétique riche et unique. En outre, cinq haplotypes principaux ont été construits sur la base des variants dans les gènes de la caséine (*CSN1S1-CSN2-CSN1S2-CSN3*), et les haplotypes B-A1-A-B, B-A1-A-A et C-A2-A-B étaient les plus fréquents.

Enfin, la publication 5 rapporte l'utilisation ou l'importance agricole et écologique des bovins Dahomey dans les pays européens. Cette race correspond à la race bovine béninoise Lagunaire, qui a été introduite en Europe au début des années 1900. Une grille de question semi-ouverte préparée en ligne a permis d'investiguer les motivations et les pratiques de gestion des éleveurs

qui ont adopté la race bovine Dahomey en Allemagne, en Suisse et en Autriche. Les résultats ont révélé un intérêt croissant pour les bovins Dahomey, qui étaient principalement élevés en petits troupeaux (sept animaux en moyenne) pour l'entretien du paysage, la production de viande ou la reproduction. Les agriculteurs apprécient diverses caractéristiques (résistance aux maladies, facilité de vêlage, faibles besoins alimentaires, qualité de la viande, etc.) des bovins Dahomey et se consacrent à la promotion de la race.

Dans l'ensemble, cette étude a mis en évidence une forte corrélation génétique entre les caractéristiques adaptatives et productives des bovins indigènes d'Afrique de l'Ouest au Bénin. Les résultats actuels corroborent les connaissances antérieures sur le bétail indigène d'Afrique de l'Ouest ou d'autres pays africains. Leurs implications pour la gestion durable des races ont été largement discutées dans ce document. D'autres recherches visant à consolider ces résultats et à améliorer la gestion des ressources génétiques des bovins ouest-africains ont été également suggérées.

1. General Introduction

1.1. Background

African cattle population is comprised of a rich diversity of breeds with unique and valuable characteristics. More than 150 national, transnational and regional taurine (*Bos Taurus*), Zebu (*Bos indicus*) and crossbreeds are reported across the continent [1–3]. In addition, many other breeds remain undescribed or are considered as extinct [3–5]. African cattle breeds are the results of thousands of years of domestication, migration, natural selection due to adaptation to diverse agro-ecological conditions, artificial selection and crossbreeding [6,7]. For instance, diverse cattle breeds have survived to diverse natural catastrophes including endemic diseases (e.g. vector-borne diseases such as trypanosomiasis, foot and mouth disease, Anthrax), and long-term droughts (exposure to heat, feed and water scarcity) [2,8]. They have acquired valuable adaptive traits such as resistance to diseases and food shortage [9]. Simultaneously, animal selection and crossbreeding are undertaken by farmers to meet specific environmental requirements or personal breeding goals [10–12].

The variety of farmers' breeding objectives and the multi-functionality of cattle sustain the diversity of the breeds in the continent [13]. Cattle play various socio-cultural, economic, environmental roles and make significant contributions to food security [14,15]. Indeed, they are acknowledged for their cultural value and significant role in the livelihood system in Africa [1]. They are sources of social status and prestige, and used in socio-cultural events including funerals and sacrifices [16,17]. Cattle also serve as productive assets, sources of revenues, forms of saving and means of insurance for millions of farmers across the continent [8,18]. Moreover, the integration of cattle production to crop farming as well as the exploitation of cattle in areas that are not suitable for crop farming, increase their values in Africa [19,20]. Unfortunately, the cattle breeding sector remains undeveloped and unable to meet the increasing challenges of food insecurity and poverty in Africa. Public policies and financial investments in the livestock sector generally overlook the complex interaction between environmental, economic, social requirements, which characterizes low-input farming systems in African countries [19]. In consequence, they fail to meet the needs of the majority of cattle farmers [21]. Ouédraogo et al. [22] indicated the lack of an adequate definition of breeding objectives and insufficient implication of smallholders as major limitations of breeding programs in West Africa. Similarly, Marandure et al. [19] reviewed cattle development programs in South-Africa and reported insufficient socioeconomic and environmental outcomes.

Many breeding programs across the continent generally focus on increasing milk yield or body size [23,24]. In this regard, crossbreeding with European or Asian breeds is common [17,25,26]. The focus on exotic high-productive breeds in livestock intensification and breeding programs in the continent has marginalized indigenous African cattle breeds [21]. These breeds are generally considered as low productive. Yet, African cattle breeds remain poorly characterized, but display enough evidence for efficient productive performance under harsh conditions [27]. For instance, some breeds including the Butana in East Africa, Azawak and White Fulani in West Africa present good dairy performance [3,28]. Other breeds such as the Gudali, Kenana and Sanga also show sufficient growth performance [3,29]. In addition to milk and meat quantity, the indigenous cattle breeds are also described for the quality of their products [30]. Furthermore, they present good reproductive performance (fertility) in addition to their higher resistance to diseases. These adaptive and productive features are increasingly evidenced by diverse genetic analyses. Genome-wide association studies (GWAS) demonstrated the association of functional and performance traits (e.g. animal growth and health) in African breeds with known quantitative trait loci (QTL) [31,32]. Similarly, several selection signature analyses in African breeds reported genomic footprints in regions associated with adaptation to diseases and heat as well as growth and milk traits [33–35]. Researchers also target specific genes including milk proteins genes and observed a large variation in African indigenous breeds [36–38]. These findings confirm the imperative need to document the potential of African genetic resources through phenomic and genomic research, and to initiate adequate selection and improvement strategies [39–41].

West African animal genetic resources include a large diversity of breeds including the shorthorn taurine (e.g. Muturu, N’Dama, Baoulé or Lobi), the Zebu (e.g. M´Bororo, Gudali) and crossbreeds (e.g. Kuri) [5,42]. The N’dama breed is the most frequently considered in scientific investigations [43–45]. These longhorn taurine cattle are involved in breeding programs in different countries [22]. However, they differ from many other indigenous breeds, which have received so far very little research and policy attention. The later breeds are also barely considered in breeding programs. Only very few examples exist handling the Azawak Zebu, Zebu Fulani and Baoulé [22].

In Benin, indigenous West African cattle genetic resources consist of the shorthorn taurine Lagune and Somba and the crossbreeds Borgou and Pabli [46]. The Lagune was exported from Benin during the beginning of the 1900’s, and is currently kept as Dahomey cattle in some African and European countries [42]. The Beninese indigenous breeds are also marginally addressed by phenotypic and genotypic characterization, which are the basis for sustainable use of animal genetic resources (AnGR). Simultaneously, the ongoing climate pressure with subsequent disease occurrence and changes in management systems threaten the continuous use of these valuable genetic resources [47–49]. Moreover, the continuous mobility of pastoralists from neighboring Sahelian countries and their settlements in Benin increase the risk of genetic dilution through crossbreeding with indicine breeds [50]. In this regard, several authors [8,51–53] have warned about the decline of the Beninese cattle populations (especially the Somba and Lagune) due to high mortality, feed and water shortages, poor reproduction, crossbreeding and lack of supportive policies. Therefore, it is urgent to characterize indigenous cattle breeds of Benin, and increase scientific knowledge on their adaptive and productive features, in order to promote their preservation, improvement and sustainable management.

1.2. Objectives and Structure of the Study

The current study aimed to genetically characterize Beninese indigenous cattle breeds by examining the genetics of their adaptive and productive features. Specific objectives targeted: i) the genetic diversity of Beninese cattle breeds, including the Dahomey cattle, in their current agricultural utilization; ii) genomic regions under selection in Beninese cattle breeds, resulting from adaptive responses to environmental factors including changes in livestock management systems; iii) genomic polymorphisms in Beninese indigenous breeds and their associations with morphometric traits; and iv) specific milk protein variants and casein haplotypes in Beninese indigenous breeds.

These objectives are addressed in five scientific publications included in the current study. They follow this general introduction, which presents the background, objectives and outline of the study.

Publication 1 reviews cattle genetic resources and their current management with respect to livestock husbandry in Benin. In addition, various approaches and strategies for the improvement and sustainable use of Beninese cattle breeds are explored.

Publication 2 focuses on the genetic characteristics of Beninese cattle populations. Current and historical genotype data are contrasted to determine the admixture process, inbreeding and recent selection signatures in the Lagune, Borgou, Pabli and Somba breeds. In addition, genetic differentiation resulting from the isolation of the Dahomey cattle is assessed.

Publication 3 investigates the genetic architecture of animal growth in Beninese indigenous cattle breeds. For this purpose, genetic parameters were estimated and genome-wide associations were performed for six morphometric traits (height at withers, sacrum height, heart girth, hip width, body length, and ear length) using medium SNP marker data from four breeds sampled in eight agro-ecological zones of Benin.

Publication 4 examines polymorphisms in the milk protein genes *CSN1S1*, *CSN2*, *CSN1S2*, *CSN3*, *LALBA*, and *LGB*. Next-generation sequencing is performed to identify known and unknown SNP, insertion and deletion (InDel). The identified variants are then used to determine milk proteins variants and casein haplotypes in the breeds. Moreover, potential functional effects of the polymorphisms on gene functions are evaluated *in silico*.

Publication 5 reports the agricultural and ecological worth of Beninese indigenous cattle outside the country. In this respect, breeding objectives and interests of European farmers as well as the management systems of the Dahomey cattle in three European countries are investigated.

Finally, a general discussion describes the major implications of the findings in the study, and perspectives for sustainable management of Beninese cattle breeds.

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2. Scientific Publications

Publication 1. **Sustainable Management of Animal Genetic Resources to Improve Low-Input Livestock Production: Insights into Local Beninese Cattle Populations**

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Review

Sustainable Management of Animal Genetic Resources to Improve Low-Input Livestock Production: Insights into Local Beninese Cattle Populations

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Abstract: Persistent food insecurity, along with climate change pressures, urges on the definition of suitable strategies to support the transformation of low-input livestock farming in developing countries. Advances in genomics and information technologies are valuable opportunities for achieving the required improvement, but their implementation implies access to technical and financial resources with appropriate adjustment in the local context. Addressing extensive cattle production in Benin, we focus on animal genetic resources (AnGR) and corresponding approaches aiming for a durable breeding improvement based on sustainable management strategies. Specifically, smallholder breeding goals are revised, and novel strategies for proper organization, sound data collection, and scientific investigations are explored. Further emphasis addresses the enhancement of farmer management practices, including valuable traditional knowledge; and breeding strategies to improve animal performances considering robustness and further traits favored by the farmers. Against such a background, it is imperative that political, institutional, and scientific collaborations reinforce research capacities and technical and financial resources. In the given context, the current review article provides policymakers and national and international researchers with practical guidance based on scientific criteria for a smallholder livestock amelioration in Benin, scalable to other countries in sub-Saharan Africa.

Keywords: smallholder cattle farming; genetic improvement; indigenous breeds; breeding goals; community-based breeding program; climate change

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1. Introduction

Agricultural production, especially livestock, in developing regions is characterized by a high vulnerability, which is intensified by diverse challenges, including climate change [1]. Increasing temperatures and humidity stimulate further environmental stressors, such as the occurrence of infectious and non-infectious cattle diseases or seasonal fluctuations in feed resources. Hence, climate change poses serious risks to the sustainability of livestock systems and threatens the livelihoods of hundreds of millions of poor livestock farmers [2–5]. Simultaneously, people in sub-Saharan Africa are highly affected by a deficit in food supply, implying continuously increasing demand for livestock products due to rapid urbanization and population growth [6,7]. The increasing challenge to ensure food security combined with the uncertain future in developing countries suggest strengthening animal genetic resources (AnGR) that are able to survive, grow, and reproduce in harsh environments. Researchers attributed the current resilience of the African pastoralism to the outstanding experiences of the herders in maintaining and managing local breeds [8–10]. African indigenous breeds have adapted to diverse, stressful tropical environments by undergoing, over centuries, natural and artificial selection. They have acquired a range of unique adaptive traits, such as resistance to disease and heat, tolerance to water scarcity, and ability to cope with poor-quality feed [11,12]. However, such a unique pool of AnGR is threatened with extinction while the development of breeding strategies considering smallholder management practices is neglected [4,7,13,14].

The need to conserve biodiversity for food production is acknowledged worldwide nowadays, but strategies to use African AnGR for improving livestock productivity and sustaining smallholder livelihoods are not thoroughly elaborated [8,15–17]. Consequently, several livestock development programs on the African continent have failed [18–20]. In addition, efforts to exploit up-to-date technologies for improving low-input livestock farming are unevenly observed across the continent. There are only a few reports from the eastern and southern African regions or Sahelian countries where the livestock production sector is more dynamic and represents a national priority [19,21,22]. The existing proposals and recommendations in this regard are not directly applicable or transferrable to various areas due to the complexity and diversity characterizing African farming systems [7]. The current situation indicates the necessity to contextualize livestock development schemes within the framework of livestock keepers' smallholder preferences and practices, as well as local economic and ecological environments [23].

In Benin, strategies for improving smallholder cattle production are very limited, whereas cattle production is vital for reducing poverty and the deficit in animal protein for the growing population in the country. In comparison with other animal species (poultry, sheep, goat, pigs, etc.), smallholder cattle herds are the main source of meat in the country, contributing to more than 50% of the national meat production [24,25]. They provide not only food but also regular revenue and social security assets for poor farmers as well as for numerous people involved in the meat, cheese, and animal trades [26–28]. Cattle farming represents a main rural activity in several Beninese agro-ecological regions and is increasingly integrated into crop farming for draught or to improve soil fertility [14,29]. The importance of cattle production in Benin is continuously growing due to the increasing movements and settlements of several pastoralists from neighboring countries [30]. In parallel, the intranational and cross-border mobility of pastoral herds is significantly affecting local animal genetic and pastoral resources [31]. These trends are expected to increase in the coming years, considering the advancement of the Sahelian desert towards the northern regions of Benin, as an evident effect of climate change [13,29]. Therefore, it is urgent to develop resilient breeding systems to increase cattle productivity and to mitigate the impacts of ecological pressures on smallholder livelihoods [7,32].

Capitalizing on the existing knowledge of Beninese cattle production systems along with the prevailing challenges [14,33,34], we review current literature and recommendations on livestock development, management of AnGR, and breeding programs to propose a framework for improving smallholder livestock keepers' livelihoods in Benin through sustainable management of their cattle genetic resources and improvement of livestock production.

2. Genetic Resources in the Context of Cattle Production in Benin

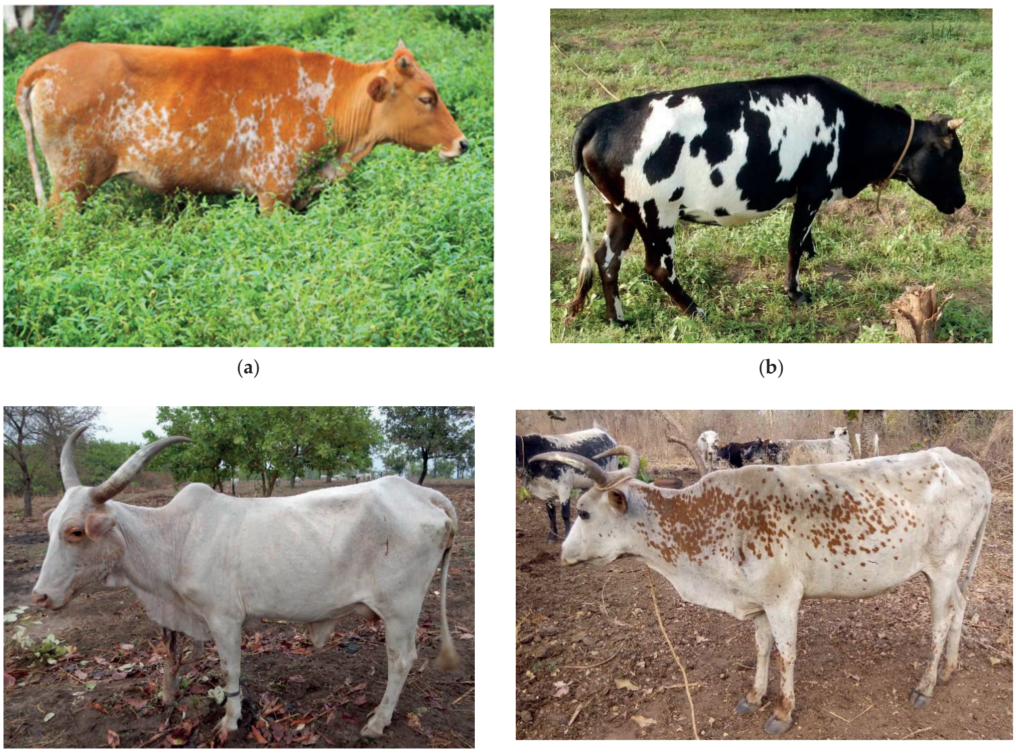
2.1. Diversity of Cattle Breeds in Benin

2.1.1. The Indigenous Cattle Breeds of Benin

Cattle production in Benin is based on two indigenous shorthorn taurines (Somba and Lagune) and a large diversity of indigenous hybrids, including the Borgou and Pabli (Table 1) [35].

The unicity and indigenous status of the shorthorn taurine are supported by their exclusive presence in West Africa since 2500 BC [36,37]. These breeds acquired their current trypanotolerance through genetic adaptation to trypanosomiasis pressures in the rainforest regions of central and western Africa [36]. The savannah shorthorn taurine cattle in Sudano-Guinean savannahs regions diverge from the dwarf (forest) shorthorn populating the coastal regions of West Africa with noticeable phenotypic and genetic differences [31,36].

In Benin, savannah shorthorns are represented by the Somba cattle (Figure 1a) distributed in the hilly region of Atacora in Benin and Togo [38]. It is reported that the Somba cattle originated from north-central Nigeria and constitute the founder of Baoule or Lobi in Ghana, Burkina Faso, and the Ivory Coast, where they migrated later [36]. The Lagune breed (Figure 1b), the Beninese dwarf shorthorn taurine, is described as the shortest cattle breed in the world [36]. Its small body size is attributed to adaptations to subsistence farming systems in the region [36,39]. Lagune cattle from Benin, known as Dahomey cattle, are reported in diverse countries in Africa (Congo, Zambia, and Gabon) and Europe (Germany, Austria, Czech Republic, and Switzerland) [36,40]. The production environment, phenotypic characteristics, and reproductive performances of Somba and Lagune cattle under extensive management systems are extensively described in recent studies [33,41–44]. Both Somba and Lagune breeds are well appreciated by farmers for their ease of management, resistance to diseases, tolerance to feed and water shortages, and good milk and meat qualities [33,44]. The breeds are also well valued for their sociocultural functions and their contribution to farmers' livelihoods, including integration to crop productions and means of saving or insurance [38]. They are also preferred in markets and fetch higher prices (per kilogram of live weight) compared with other breeds, such as the Zebu [26]. Nevertheless, their small size and low productivity limit their continuous management, as they are continuously replaced by crossbreeds with Zebu cattle [31].



(**a**) (**b**)

(**c**) (**d**)

Figure 1. Photos of Beninese indigenous cattle breeds: (**a**) Somba, (**b**) Lagune, (**c**) Borgou (crossbreed Somba × Zebu White Fulani), and (**d**) Pabli (crossbreed Borgou × Somba).

According to genetic analyses (formal tests of admixture and estimation of ancestry proportion), the Borgou breed (Figure 1c) is originally a product of crossbreeding between the shorthorn Somba cattle and the Zebu White Fulani [45]. The Borgou are characterized by higher meat and dairy performances than those of the taurine cattle, and they have adaptive abilities, including tolerance to trypanosomes. In the past, the Borgou was a favorite smallholders' breed given its meat and milk (organoleptic and technological) qualities (over Zebu or other exotic breeds) [46]. No national breed census exists to date, but the breed was reported as the largest cattle population in Benin according to different studies [14,47]. However, the Borgou are stepwisely crossed with Zebus, aiming for higher productivity [31]. Similarly, the Pabli (Figure 1d), a crossbreed between the Borgou and Somba, were described in northwest Benin [48,49]. The status of this breed is uncertain, while diverse products of crossbreeding are increasingly reported in Benin cattle herds. The expanding admixture in the national cattle herds due to both intended and unintended crossbreeding with less adapted Zebu and crossbreeds questions the sustainability of cattle production in the country and requires urgent actions.

2.1.2. Other Cattle Breeds Reared in Benin

Several west and central African Zebu cattle breeds supplement the diversity of cattle breeds in Benin (Table 1) [35]. The Zebu breeds, essentially present in Sahelian and other West African countries, have been introduced in Benin through transhumance movements and pastoralist settlements [30,35]. Generally, Zebu cattle are less adapted to the Beninese humid climate than the taurine cattle are, but some Zebu breeds, including the White Fulani cattle, have appreciable adaptive features. Historians and geneticists explain the relative adaptability of Bororo cattle (White Fulani and Red Fulani) by their crossbreeding origins from *Bos primigenius* and *B. indicus* [10,50]. White Fulani cattle are predominant in Benin and are widely used in crossbreeding, as they are simultaneously suitable for draught and milk and meat productions [51]. In addition to Bororo cattle (lyre-horned), shorthorn Zebus are also described in Benin and are represented by the Goudali and Azawak breeds [35,52,53]. The latter Zebu group is originally described as “pure Zebu”, with large size and high productive performances [10,54–56]. A major limitation of these breeds addresses their demanding feed requirements [10]. Nevertheless, the crossbreeding of the Azawak with indigenous breeds is currently promoted in extensive, as well as state-owned, farms [53,57]. To date, there is a lack of scientific evaluation addressing the characterization of these crossbreeds and their adaptation to Beninese local conditions. Further Zebu breeds in Benin include the Djeli or Nigerian Fulani, which is mainly reported in Niger [35,58]. However, the low resistance or tolerance of this breed to trypanosomiasis limits its expansion in Beninese pastoral regions [14,52]. The presence of Zebu cattle in several West African countries and the involvement of some breeds in ongoing breeding programs offer possibilities and perspectives for regional collaborative programs [19].

In addition to the Zebu, the N’Dama and Girolando are also reported in Benin [59,60]. The Longhorn Taurine N’Dama cattle were introduced on the Okpara state-owned farm in north Benin in 1952 and 1993 in order to improve Beninese indigenous cattle breeds [61]. The N’Dama presented lower productivity than that of the Borgou in Benin and has not been successfully promoted into extensive farms [62]. Consequently, the current N’Dama population exhibited inbreeding depression on the Okpara state-owned farm [63]. The Girolando, a crossbreed from the Asian Zebu Gyr and the Holstein, was first introduced in 2004 for the increase of national cattle milk productivity in Benin [59,60]. The breed is known for high milk performance and adaptation to tropical conditions, including tolerance to heat stress [64]. However, the Girolando cattle are less adopted by smallholders in Benin. They are less productive than expected due to their low adaptation to local productive conditions, including high disease pressures and limited availability of feed resources [65]. In addition, the Girolando are suspected as a source for the spread of new invasive tick species (e.g., *Rhipicephalus microplus*) in Benin, as in other West African countries where the breed has been introduced [66–68].

Table 1. Characteristics of the cattle breeds in Benin.

Category	Breed Name *	Presence in Benin #	Geographical Distribution in Africa	Frequently Desired Attributes by Farmers	Weakness	Observations	References
Savannah shorthorn	Somba	Northwest Benin (Boukombe)	Northeast Togo	Tolerance to diseases and feed and water shortages; good milk and meat qualities; good temperament and draught ability; ease of management; high sociocultural value	Small size, low milk and meat productivity	Reduction of population size due to diseases and lower effect of admixture	[31,33]
Dwarf shorthorn	Lagune	Southern and central Benin	Congo, Zambia and Gabon (known as Dahomey cattle)			Moderately affected by admixture with Zebu	[31,43]
Indigenous crossbreeds	Borgou	Across the country	Known as Ketekou or Keteka in West Africa	Tolerance to trypanosomiasis and feed and water shortages; endurance and draught ability	Lower milk and meat productivity than the Zebu	Highly affected by admixture with Zebu	[14,31,35,52]
	Pabli	Northwest Benin (Kerou)	-	Share similar characteristics with the Borgou	-	Less investigated and reported as extinct	[31,69]
Lyre-horned Zebu	White Fulani or White Bororo [1]	First most reported Zebu cattle in Benin	Central and western Africa	More resistant to diseases and tolerant to heat than other Zebu are; good performances in milk, meat and draught	Exigent in feed and water resources: practice integral and low grazing.	Large diversity and admixture within the population Its adaptive features are barely investigated	[10,36,51,70–72].
	M'Bororo or Red Fulani [2]	Third most reported Zebu cattle in Benin	Central and western Africa	Ability to walk long distances; intelligent animal, docile and attached to its owner (less susceptible to theft)	Less trypanotolerant, very exigent in feeding: practice selective grazing	Poorly investigated for population genetic characterization	[10,72–74]
Shorthorn Zebu	Gudali [3]	Second most reported Zebu cattle in Benin	Nigeria, Ghana, Cameroon, Central African Republic, and Mali	Large size, growth, and milk performance; exploits large variety of feed resources in the dry season	Poor carcass yield: less than that of White Fulani Limited in walking	Large genetic diversity: many subpopulations; current distribution unknown in Benin	[52,54–56,72,75,76]
	Azawak [4]	Very rare in extensive herds and crossbreed in state-owned farms	Mali, Niger, and Nigeria	Best milk performance within indigenous WA cattle, good meat performance, and excellent adaptation to drought	Requires high-quantity and qualified feed; slow in walking long distances	Currently promoted for crossbreeding with the indigenous breeds in extensive and state-owned farms The breed and resulting crossbreeds remain largely undescribed	[53,56–58,77]
Undescribed (crossbreed) Zebu	Djeli or Nigerian Fulani [5]	More rare than other Zebu	Niger	Good reproductive and milk performance, weight gain, and docility	Less trypanotolerant, with medium size and body weight	Remains largely undescribed No study on genetic diversity and relationship with other breeds	[52,58,77]
Longhorn African taurine	N'Dama	Mainly kept on state-owned farms	Western and central Africa	Trypanotolerance and resistance to diseases	Less productive than the Borgou and Zebu are	High inbreeding in the current population on state-owned farms	[61–63,72,78]
Tropical crossbreed	Girolando	Kept on Kpinnou and Okpara state- owned farms	Ivory Coast, Burkina Faso, and Senegal	High milk performance	Very susceptible to disease pressures and exigent in feed and water resources	Low productivity in Benin due to inadequate environmental conditions and source of tick invasion in West Africa	[60,66,67,79,80]

* Others breed names: [1] Yakanaji, Daneeji, Akuji, or Bunaji [51,81]. [2] M'Bororo, Bororo, Mbororooji, Bodeeji, or WoDaaBe [35]. [3] Goudali, Sokoto Gudali Bokoloji, Rahaji, or Zomanta [35,81]. [4] Azaouak, Azawa, Azawaje, Tuareg, Adar, Darmeghou, or Tagama [56,82]. [5] Djelli, Djelliji, Diali, Djalli, Jaliji, or Peuhl Nigérien [35,56]. # There is to date no official statistics on the population size of cattle breeds in Benin. The information presented here is based on systematic surveys from previous publications.

2.2. Management of Cattle Genetic Resources in Benin

2.2.1. Cattle Production Systems and Major Constraints

In Benin, cattle are mainly kept in low-input extensive farming systems, as intensive or commercial herds are uncommon. The major production systems comprise sedentary and mobile herds. Sedentary herds are of small size, dominated by indigenous taurine cattle, and principally rely on village pastures the whole year for feeding [14,83–85]. Mobile herds are larger and mainly owned by traditional pastoralists (Peulh, Fulani, Fulbe, MbororooBe). They seek feed and water resources within and beyond their principal encampments or regions through low- or high-amplitude transhumance or migration [14,83,86].

The shortage of feed and water resources represents a major limitation for cattle farming [87–89]. The distribution and availability of feed and water resources are driven by a high variability of climatic conditions across the country [47,90,91]. Simultaneously, the majority of pastoral rangelands and transhumance corridors have faced intensive degradations due to overgrazing and bush fires. Rangelands and transhumance corridors are frequently replaced by crop farms (especially cash crops, such as cotton), which have been increasingly expanded in pastoral regions. The competition in land use resulting in reduction of pastoral rangelands is enhanced by demographic explosion, social considerations, and land property rights [92,93]. Simultaneously, the delimitation and management of pastoral resources by local or national authorities, as well as their scientific characterization, are barely observed [94,95].

High rates of mortality and disease occurrence constitute the second main constraints in the Beninese cattle herds [14,33]. Major diseases (several are neglected zoonoses) reported in Benin include viral (foot-and-mouth disease and lumpy skin disease), bacterial (anthrax, contagious bovine pleuropneumonia, and *Mycobacterium tuberculosis*) and parasitic (trypanosomiasis and *Fasciola gigantica*) infections [33,65,96–102]. The extensive systems, grazing on communal pastures, and national and cross-border herd mobility and trade favor the spread of diseases in the agro-pastoral areas [27,101,103–105]. The diagnosis and monitoring of livestock diseases are limited, and prevalence data are scarce. The treatment and prevention of disease through vaccination are also deficient, being based mainly on self-medication with medicinal plants or on important quantities of antibiotics and trypanocides [106,107]. According to Dognon et al. [106], animals are generally over- or underdosed because animal body weights are not correctly estimated and farmers have no expertise in drug usage. The scarcity of professional assistance in the treatment of the disease increases drug resistance, increases mortality in cattle herds, and impairs the quality of cattle products [108,109]. For instance, researchers reported more than 68% of resistance to the *Diminazene aceturate* trypanocide in West African cattle herds [109].

2.2.2. Institutional Management of AnGR in Benin

In recent years, noticeable efforts have been made regarding the characterization of production systems and phenotypic characterization of Beninese cattle breeds [14,33]. Nevertheless, there is a lack of knowledge addressing cattle production and the description of AnGR in Benin. The genetic characterization of AnGR is quite unexplored in Benin. Only a few studies have revealed a high level of diversity and quite large genetic distances between the different populations [31,45,53,110,111]. Considering the existing genomic studies [31,45,53,110,111], we assume only a thousand local animals were genotyped (with microsatellites and 50 K Illumina SNP), which is a very small fraction in relation to the whole national cattle population [24]. Research activities on AnGR are limited by logistic constraints in data collection related to the extensive production system (including herd mobility), challenging conditions in pastoral areas (farms are hardly accessible and no electrical energy sources on field), absence of national data collection systems, and the lack of qualified technicians, adequate equipment, and infrastructures to collect valid data in the field [41,112,113]. In addition, the involvement of the herders in the development and implementation of research activities is insufficient.

The last national assessment for the “second report on the state of the world’s animal genetic resources for food and agriculture” undertaken by the Food and Agriculture Organization (FAO) [16] described a poor institutional context for the management of AnGR in Benin. This is characterized by the absence of national legislation, suboptimal breeding programs or strategies (including artificial insemination breeding), a deficit of relevant infrastructure, poor education, training, and collaboration between the stakeholders (farmers, authorities, traders, and consumers), and a lack of public knowledge and awareness regarding the management of AnGR [114]. This diagnosis contrasts with the reports of the numerous and diverse livestock development projects (PDE I, II, III and PAFILAV) implemented in the country since the 1980s [59,115]. These projects aimed at “modernizing” livestock production in Benin (by addressing livestock feeding, health improvement, genetic improvement, and animal product processing and marketing) have generated very few impacts [60,115]. A typical example is the Milk and Meat Support Project (PAFILAV) targeting the enhancement of the national productivity in cattle milk and meat through crossbreeding with Girolando cattle from Brazil [60]. The generated crossbreed animals barely survive disease pressures, resulting in low productivity despite their controlled management systems [60,79]. The Girolando crossbreeds, like other publicly imported breeds (e.g., N’Dama), are generally kept on state-owned farms and are hardly adopted by smallholders for reasons including fitness and product quality [46,78]. These examples indicate the critical necessity for a paradigm shift and the conception of relevant strategies adapted to local environmental conditions and the needs of smallholders.

3. Pathways for the Management and Improvement of Cattle Genetic Resources in Benin

3.1. Livestock Development Objectives and Strategies

3.1.1. Breeding Goals of Smallholders

The demand for milk and large-sized cattle in Benin is growing continuously and is secured by the importation of meat and dairy products [44,60]. However, targeting for breeding objectives and breeding goals that only include meat and milk productivity, is usually ineffective, as observed in diverse African countries [18]. Indeed, smallholders perceive the improvement of their herds’ overall productivity in a more complex system than that only focusing on increasing growth and milk performances. Previous studies indicated that smallholders are mainly concerned with the maintenance of their herds as insurance or productive assets for primary household consumption and a source of regular or diversified familial income [116,117]. Therefore, the definition of specific and nonclassical breeding goals, including farmer objectives and their desires of ideal animals, is fundamental for any promising livestock improvement strategy (Figure 2) [118,119].

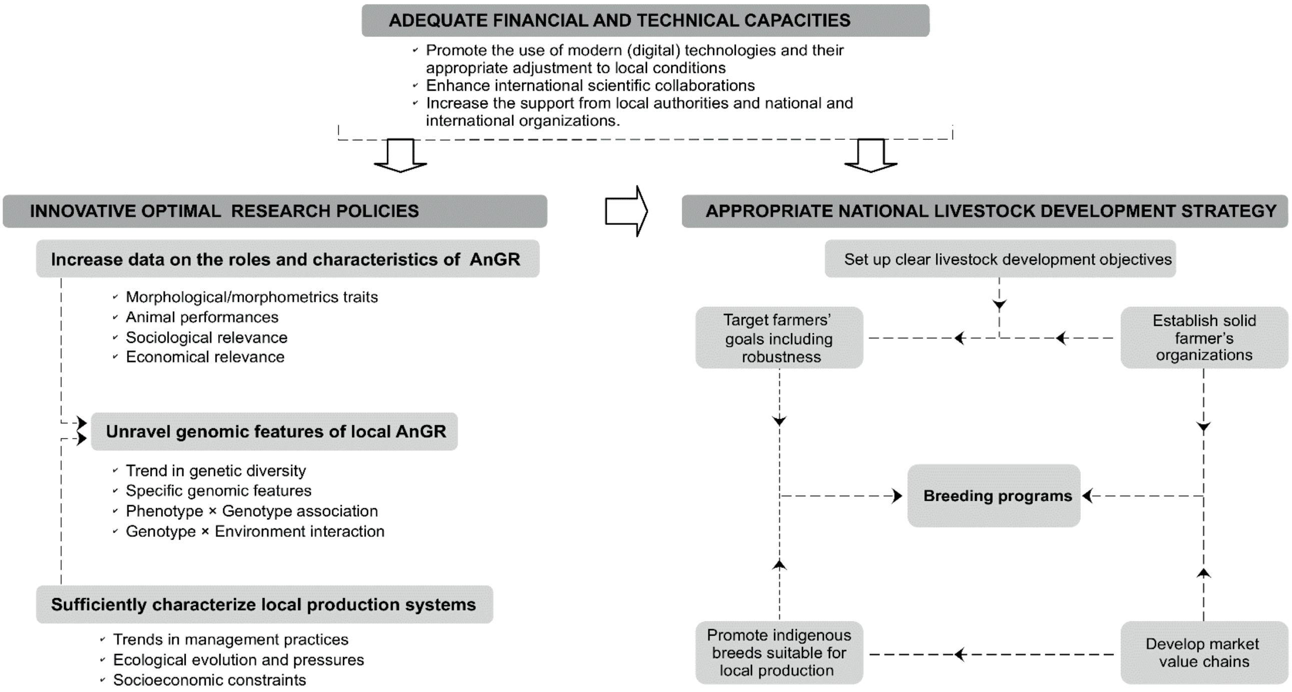


Figure 2. A holistic scheme to address the management and improvement of animal genetic resources (AnGR) in Benin.

Smallholder perceptions of a breed and their decision to keep or "create" a breed are driven by personal interests, sociological and historical backgrounds, production objectives, production system, and environmental constraints. Although high diversity in herder interests and breeding objectives is observed in Benin and other African countries, major distinctions between the agro-pastoralists and mobile pastoralists address sociocultural characteristics and management systems (Table 2). In Benin, smallholder agro-pastoralists (practicing cattle farming as second activities) mainly value the sociocultural functions of their animals and their utility in cropping activities [38]. These farmers principally focus on draught ability, manure, morphological appearance (such as specific coat color for cultural purposes), and animal docility. They prefer animals that are robust and easy to keep, which may be defined by adaptive traits such as resistance to disease, tolerance to feed, and water shortages or animal longevity [33]. In contrast, mobile pastoralists favor animal fertility, as the breeding of large cattle herds and milk production are essential components of their culture and the basis of their social and wealth status [120]. The mobile pastoralists are more oriented toward milk yield and quality, as well as growth performance. However, adaptive traits are also important, especially with the worsening agro-ecological conditions. Boutrais [76] reported that mobile pastoralists first created a new "crossbreed" in order to adapt to new environmental conditions, but they upgraded this new crossbreed to consider the characteristics of their ideal cattle (large conformation and high milk productivity). The later observation indicates that interests of farmers are not static and may be influenced by sociological, economic, and ecological constraints. Therefore, an exhaustive and regular assessment of farmers breeding goals would be required.

Table 2. Major farmer preferences and breeding objectives generally observed in African low-input cattle production systems.

	Agro-Pastoralists	**Mobile Pastoralists**
Production	Meat quality Draught ability	Live weight Milk yield Milking easiness Milk quality (cheese making)
Reproduction	Calving ease Calf survival	Fertility (short calving interval) First calving at young age
Robustness	Ability to produce with limited feed and water (quantity and quality) Resistance to diseases (Trypanosomiasis) Longevity	Moderate exigence in feed and water Ability to walk long distances Tolerant to heat stress
Temperament	Docility Ease of keeping	Aggressiveness (against theft)
External appearance	Coat color	Large conformation
References	[14,33,34,38,42,44]	[14,34,35,44,52,76,117]

3.1.2. Optimization of Phenotyping and Genotyping Strategies

Identifying the breeding goals of farmers and determining suitable breeds for local conditions require the collection and analysis of large-scale and accurate animal phenotype and genotype data in the context of extensive descriptors for environmental and management systems. The current scarcity in data and lack of initiatives in Benin contrast with the diversity of emerging tools and guidelines to support data collection and analysis in low-input livestock production systems [121,122]. This suggests the enhancement of local capacities and the adjustment of up-to-date technologies to provide local, convenient, and cost-effective but powerful strategies for optimal scientific investigations toward the improvement of livestock breeding in Benin [123].

Morphometric traits represent valuable measurements for the phenotypic characterization of AnGR, performance evaluation, and animal selection in both developing and developed countries. Recent studies in Benin reveal that they could be accurately exploited for performance recording (estimation of live body weight) and genetic evaluations [34,113]. Beside morphometric measurements, innovative phenotypes, such as worm fecal egg counts, antibody levels in response to pathogen infections, rectal temperature, respiration rate, and milk content, are valuable indicators to evaluate adaptive features, including heat tolerance, disease resistance, or tolerance [118,124].

Emerging information technology (IT) offers new prospects for systematic collection and analysis of diverse agricultural data [125]. Geographic information systems (GISs) assist in assessing environmental descriptors for genomics and breeding purposes, and experiments have been performed in North Africa [126]. In addition, ecological and epidemiological data from satellite information are freely accessible on various open-source platforms [127]. Zannou et al. [101] exploited satellite images to characterize transhumance corridors, vegetation, and risk of diseases in transhumant herds from Benin and neighboring countries. Similarly, drone technology may help to investigate livestock populations or to support the monitoring and management of pastoral rangelands [128]. In eastern and southern African countries, mobile phone and data loggers are now used to collect large-scale management, health, and performance (milk performance, morphometric traits, or body condition score) data in smallholder households and livestock herds [123,129]. The utilization of mobile phones by increasing numbers of Beninese pastoralists, as observed by Djohy et al. [129], is an attractive opportunity. In addition, the ongoing extension of mobile internet connections and emerging

IT competencies in the country are the basis for the development of similar digital applications. Good collaboration between scientists and local IT experts is therefore expected to explore and define innovative phenomics and environomics (large-scale phenotypic and environmental data capturing) systems for livestock research in Benin [130,131].

Regarding genotypic data, the availability of kits and protocols to easily and accurately extract DNA from animal tissues, such as hair samples (conveniently collectable and conservable), enabled animal genotyping in challenging breeding conditions [132]. This revolution has been supported by the development of affordable low- or medium-density single-nucleotide polymorphism (SNP) panels. The majority of commercial SNP chips are sufficient to assess the genome of indigenous breeds in Benin, but these chips are developed for commercial large-scale populations and may be less informative to establish effective genomic selection of African taurine, Zebu, or crossbreeds [133]. Hence, the development of specific medium-density chips suitable for Beninese or West African cattle breeds is highly recommended. Finally, the creation and regular updated repositories and genomic databases for Beninese and African AnGR will support adequate AnGR monitoring, enabling further advances through meta-analyses and collaborative studies [134].

3.1.3. Structured Interventions with Appropriate Farmer and Market Organizations

Clear structures and effective collaborations between different stakeholders are imperative for the organization and successful implementation of livestock improvement strategies [23,135]. Scientists have highlighted the importance of participative conception and execution of development programs with smallholders [18]. A real participation of farmers ensures effective consideration of their interests, needs, and expectations, the identification of real problems, and adequate on-herd solutions. Community-based breeding programs (CBBPs) have been proposed to promote farmers' indigenous knowledge, and ensure training, competence sharing, and institutional interventions [136,137]. CBBPs are focused on creating local interest in the management of AnGR and set up breeding and animal selection schemes within village herds [19,137]. Benin can valuably benefit from the developing expertise (in designing and implementing CBBPs) on the continent, especially in countries located in West Africa [19,137,138]. The sociocultural relationships within and between agro-pastoralists and mobile pastoralists promote the establishment of CBBPs in Benin [30,33]. For instance, the entrustment practice, where several cattle owners (generally the agro-pastoralists) place their animals under the management of a professional herder (i.e., the traditional pastoralist), is increasingly observed in Beninese pastoral areas [34,139,140]. A restructuring of existing entrusted cattle herds (gathering animals from several owners) may facilitate the establishment of village herd pools and farmer associations.

In addition to the organization of farmers in breeder associations, local operating teams that inspire trust and confidence to the participants are necessary. Marandure [18] suggested the training of young communal animal workers to support farmers, practice health services, or research activities, and to supervise the effective implementation of the respective tasks. In several pastoral villages in Benin, the participation of resident young people in breeding programs would be valuable to overcome language barriers and facilitate a good collaboration with other stakeholders. Finally, a proper breeding program design should focus on the development of local market niches, taking into account traditional structure and household organization around the management and commercialization of herd products. For instance, Chabi-Toko [117] described an organized milk management system that ensures domestic consumption, active participation of women, and fair sharing of resources among Beninese pastoralist households. This example indicates the need to involve women in cattle breeding strategies for extensive sociological and economic impacts. Moreover, the establishment of value chains that undermine the familial organization of livestock farming has very little chance of success. Evidence is the failure of recent development projects aimed at creating dairy units for gathering and processing milk from smallholders in Benin. Indeed, these dairy units are poorly supplied with milk, which does not ensure their effectiveness [141]. In addition, Beninese local milk producers are facing high competition due to imported industrial dairy products, indicating the need to develop innovative and adequate approaches for the promotion of the traditional Beninese dairy technologies and marketing [142]. Further strategies should also target the promotion of nonclassical sources of income for farmers (such as manure contracts and hiring of draught power), and the improvement of local markets with short and fair distribution systems between producers and consumers [18,143].

3.2. Breeding Programs

3.2.1. Conserving and Building on Local Cattle Genetic Resources

The long-term success of genetic improvement relies not only on the development of suitable breeds (or crossbreeds) for production but also on the maintenance of genetic diversity [20,119].

The genetic diversity within and between Beninese cattle breeds is an important asset for adaptability, sustainability, and cattle population fitness [31,144], and in consequence, is the basis for the environmental and economic viability of any improvement programs. In this regard, the conservation of Beninese indigenous breeds is a priority and consists of addressing major threats (i.e., high mortality rate, uncontrolled crossbreeding, and decreasing interest in indigenous taurine cattle) that reduce effective population size and increase inbreeding. Genomic tools offer various possibilities to evaluate the livestock population structure (considering effective population size, admixture, and inbreeding rate), to identify major threats and to apply appropriate conservation strategies [145]. Recent investigations have outlined the opportunities to promote and conserve the Somba and Lagune breeds in vivo in cooperation with local farmers [33]. Similar examples for indigenous taurine cattle, including the Baoule or N'Dama, have been reported in other West African countries [19].

Conserving Beninese taurine breeds is also important to establish backup populations for ensuring continual access to pure genetic lines in crossbreeding programs [18]. Burrow [146] recommended a minimum of 25% to 75% of "adapted genes" in breeding programs to guarantee optimal production under challenging production systems. Similarly, Knap and Doeschl-Wilson [147] suggested a breeding strategy including resistant or tolerant local animals as a cost-effective alternative to improve productivity in tropical herds. In Benin, the Borgou crossbreed represents a convenient candidate to conciliate cattle productivity with adaptability [45,148]. The current adaptability of the breed and its acceptance by many Beninese farmers imply the necessity to identify admixture rates that suit farmer objectives, breeding systems, and environmental requirements [133,135]. Furthermore, improving Zebu animals may be an alternative for some traditional pastoralists, who are very attached to these animals. The White Fulani, a three-purpose (meat, milk, and draught) cattle breed, is a recommendable breed for enhancing cattle productivity in Benin. The broad distribution of Zebu across West Africa is a chance to develop collaborative research activities and breeding programs in the subregion. Finally, the introduction of exotic breeds other than West African local breeds should represent the last resort. The use of exotic animals for crossbreeding may be explored for specific production systems (such as intensive farms), capitalizing on their advanced genetic characterization and experiences from other African countries [21,22].

3.2.2. Targeting Selection for Robust Cattle

As presented above, smallholder breeding objectives encompass a large variety of traits related to animal production, reproduction, health, temperament, and efficiency in resource use. Previous selection signatures and genome-wide associations confirm the high genomic association between productive and adaptive traits in Beninese indigenous breeds [113,144]. Therefore, defining multicomponent breeding traits considering productivity, resilience, and adaptability (instead of classical specific productive traits) is recommended for the improvement of Beninese AnGR [149,150]. The holistic breeding approach to integrate the production ability of an animal with its physiological and immunological response to environmental challenges (reaction to stress, health, feeding efficiency, etc.) is conceptualized as robustness [150,151]. A robust animal is able to produce efficiently (i.e., to maintain high performances or breeding values under variable stressors) [118,150]. The novel traits indicating robustness that are being increasingly developed in controlled dairy systems should be evaluated in the context of Beninese extensive cattle systems [124,152]. For instance, Calus et al. [152] associated robustness in dairy cattle with diverse traits (body condition, milk composition, milkability, calving interval, temperament, mastitis, and feet and leg conformations).

Moreover, advances in genomic selection contribute to enhancement of the genetic-statistical model for the estimation of variance components and breeding values considering genotype by environment interaction (G × E) [151]. In this regard, reaction norm models have been applied, studying the environmental sensitivity of a trait (e.g., productive performance) across an environmental descriptor gradient [153]. The high variability and instability of production systems and environments under Beninese conditions challenge the evaluation of G × E. However, the collection of repetitive and large-scale environmental data and the definition of suitable herd environment descriptors (such as production level, farm size, or composition, disease pressure, and reproduction management) support the investigation of G × E in a challenging production context, as observed in South Africa [150,154,155]. Furthermore, statistical Bayesian and GBLUP models adapted to small datasets enable genomic selection to achieve substantial genetic gain within a relatively short time frame for developing countries like Benin [156].

3.2.3. Improving Farmer Management Practices

Although animal selection and breeding schemes are supposed to fit local conditions and management systems, the practices of farmers need to be upgraded. Smallholders mainly expect aid from researchers or policymakers that addresses improved utilization of feed resources and the management of animal diseases. Food security and control of diseases are considered the foundation of any durable improvement in animal productivity [157]. However, the greatest attraction to avoid is the promotion of externally sourced commercial feed and veterinary inputs, being cost-prohibitive and not permanently affordable to smallholders [18].

Here too, it is important to draw on endogenous knowledge and practices to propose solutions that are accessible and easily adoptable by breeders. For instance, crop–livestock integration is described as an imperative and promising agro-ecological system to support the reduction of feed shortage and management of rangelands [158,159]. This practice is well known and has been practiced for decades in Benin. Additionally, the crop–cattle association at the household level, and manure contracts (where cattle herders exploit crop residues from other crop farmers in exchange for manure) are described in Beninese agro-pastoral areas [28,47]. The exploitation of manure is interesting in managing nutrient flows for increasing soil fertility and rangeland production [160,161]. These practices should be assessed and valorized considering previous scientific recommendations [158,162]. For the management of rangelands and water sources, various pastoralist initiatives, including better distribution of grazing areas, reorganization of herd mobility, and combination with further adaptive management practices, have been described as promising and sustainable in Cameroon [163,164]. Such initiatives should be advocated over systematic eradication of herd mobility and fodder cultivation by individual herders, which is hardly feasible in the context of land competition and difficult access to land resources by pastoralists in Benin [165]. Regarding disease prevention and treatment, traditional farming practices should be similarly supported and enhanced by scientific investigations [107]. The improvement of traditional methods for disease control associated with an improvement in the management of animal housing and feeding, as well as the breeding of resistant animals are the most sustainable solutions to avoid drug resistance and guarantee animal food quality [166].

3.2.4. Promoting Institutional Supports

The management of AnGR and improvement of cattle production are only sustainable when clear national livestock development policies, adequate institutional frameworks, relevant scientific research activities, and durable technical and financial resources are in place [17]. National authorities have the first responsibility to create appropriate sociological and legislative environments, allowing farmers to securely practice their activities and be promoted by national and international institutions or development organizations. In Benin, recent reforms have addressed the regulation of national and regional cattle mobility, as well as land use policies. However, a larger consultation with scientists and smallholders (crop farmers and livestock keepers) is required to ensure the effective and fair access of rural actors, especially pastoralists and agro-pastoralists, to pastoral resources (rangelands and watering points) [167]. Simultaneously, governmental agricultural services are expected to strengthen their interventions in rural areas. In addition, political commitment to provide incentives for scientific innovation is imperative. This implies financial support and facilities for scientific interventions [7,162]. There is a critical need to reinforce university training (especially in animal breeding and genomics) and reorganize research and development initiatives in a way that they can benefit national policies for improving the livelihoods of farmers [134]. Good scientific cooperation is fundamental to set a national research agenda oriented toward the identification of sustainable livestock development solutions. So far, collaboration between Beninese researchers has been limited, while researchers from various backgrounds (animal breeders, geneticists, system analysts, and IT specialists) are expected to develop overall data collection strategies. Datasets from different disciplines have provided an extensive comprehension of the production system and characterize the different resources involved in livestock production (AnGR, rangelands, and water sources) [18,123]. Finally, bilateral partnerships with regional and international universities and institutions will be valuable to create opportunities for the exchange of knowledge and technical resources and to enhance capacity building and research qualities. For instance, the International Livestock Research Institute (ILRI) has significantly contributed to the improvement of AnGR and livestock production in countries in eastern Africa. Such institutional supports are lacking in West Africa, especially in French-speaking countries, as the *Centre International de Recherche-Développement sur l'Élevage en Zone Subhumide* (CIRDES) entitled to support livestock production in these countries has been less dedicated to the management of AnGR [23].

4. Conclusions

Extensive cattle production has a great potential to significantly contribute to food security and enhance the livelihood of smallholders in Benin. However, the lack of adequate breeding strategies, scientific investigations, and political and financial supports is a severe constraint in a challenging environmental context including feed shortage and disease pressures. We reviewed various opportunities applicable in the current Beninese context to improve the management of local AnGR for sustainable development of low-input cattle production. We showed that appropriate strategies should be based on the breeding objectives of farmers. In addition, the adaptation of recent advances in IT, genomic applications, and statistical analyses to local conditions will help to assess the potential of indigenous AnGR and implement appropriate breeding schemes for local production systems and environmental constraints. The enhancement of local scientific and technical capacities, effective involvement of farmers in research studies, and political commitments are fundamental for durable progress. Furthermore, assistance from regional and international organizations, as well as collaboration with international scientists or research institutions, would be very valuable.

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Publication 2. **Unraveling Admixture, Inbreeding and Recent Selection Signatures in West African Indigenous Cattle Populations in Benin**

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Unraveling Admixture, Inbreeding, and Recent Selection Signatures in West African Indigenous Cattle Populations in Benin

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The Dwarf Lagune and the Savannah Somba cattle in Benin are typical representatives of the endangered West African indigenous Shorthorn taurine. The Lagune was previously exported to African and European countries and bred as Dahomey cattle, whereas the Somba contributed to the formation of two indigenous hybrids known as Borgou and Pabli cattle. These breeds are affected by demographic, economic, and environmental pressures in local production systems. Considering current and historical genomic data, we applied a formal test of admixture, estimated admixture proportions, and computed genomic inbreeding coefficients to characterize the five breeds. Subsequently, we unraveled the most recent selection signatures using the cross-population extended haplotype homozygosity approach, based on the current and historical genotypes. Results from principal component analyses and high proportion of Lagune ancestry confirm the Lagune origin of the European Dahomey cattle. Moreover, the Dahomey cattle displayed neither indicine nor European taurine (EUT) background, but they shared on average 40% of autozygosity from common ancestors, dated approximately eight generations ago. The Lagune cattle presented inbreeding coefficients larger than 0.13; however, the Somba and the hybrids (Borgou and Pabli) were less inbred (≤0.08). We detected evidence of admixture in the Somba and Lagune cattle, but they exhibited a similar African taurine (AFT) ancestral proportion (≥96%) to historical populations, respectively. A moderate and stable AFT ancestral proportion (62%) was also inferred for less admixed hybrid cattle including the Pabli. In contrast, the current Borgou samples displayed a lower AFT ancestral proportion (47%) than historical samples (63%). Irrespective of the admixture proportions, the hybrid populations displayed more selection signatures related to economic traits (reproduction, growth, and milk) than the taurine. In contrast, the taurine, especially the Somba, presented several regions known to be associated with adaptive traits (immunity and feed efficiency). The identified subregion of bovine leukocyte antigen (BoLA) class IIb (including *DSB* and *BOLA-DYA*) in Somba cattle is interestingly uncommon in other African breeds, suggesting further investigations to understand its association with specific adaptation to endemic diseases in Benin.

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Overall, our study provides deeper insights into recent evolutionary processes in the Beninese indigenous cattle and their aptitude for conservation and genetic improvement.

Keywords: Dahomey cattle, genomic inbreeding, admixture, haplotype, BoLA genes, adaptation

INTRODUCTION

Western Africa represents a reservoir of the unique and diverse African animal genetic resources, due to a complex history including migration, dispersion, natural and artificial selection, and crossbreeding (Hanotte et al., 2002; Stock and Gifford-Gonzalez, 2013). The region is the exclusive current habitat for indigenous taurine cattle (*Bos taurus*) on the continent (Mwai et al., 2015). African humpless taurine cattle are the earliest known cattle populations on the continent according to historical and archaeological evidence (Blench and MacDonald, 1999). These animals share early common ancestors with European taurine (EUT) cattle dated to before the domestication process (Ho et al., 2008; Murray et al., 2010). They are subdivided into two subgroups, the Longhorn and the Shorthorn taurine. The origins of the two populations are still controversial, but it is scientifically accepted that they have been separately introduced into West Africa several millennia BC (Epstein, 1971; Payne and Hodges, 1997; Hanotte et al., 2002). The N'Dama is the unique reported Longhorn taurine breed, whereas the humpless Shorthorns include the Savannah breeds (Baoulé, Doayo, and Somba) and the Dwarf forest breeds (Dwarf Muturu, Liberian Dwarf, and Lagune), which are barely characterized across West African countries (Rege, 1999).

The Somba and Lagune represent indigenous African Savannah and Dwarf taurine in Benin, respectively. Both are characterized by stocky animals, resistant to diseases (especially trypanosome), and able to survive and produce in harsh environments (Dossa and Vanvanhossou, 2016; Ahozonlin et al., 2020). The Somba cattle are the typical ancestral residue of West African Savannah Shorthorns that migrated to Togo, Ghana, and the Ivory-coast (Rege, 1999). They have been preserved from admixture until recent past in the Atacora mountain area in Northwest Benin (Hall et al., 1995; Rege, 1999). The Lagune cattle are present not only in Southern Benin but also in the coastal areas and near lagoons in West and Central Africa, as indicated by their name (Rege et al., 1994). The Lagune are described as the smallest of the African taurine (AFT) cattle (93 cm of average height at withers) and acquired their Dwarf phenotype through adaptation to environmental constraints in their belt (Rege et al., 1994). According to previous studies, this breed is genetically different from other Shorthorn cattle breeds (Moazami Goudarzi et al., 2001; Gautier et al., 2009). Berthier et al. (2015) reported higher trypanotolerance with lesser anemic condition in Lagune animals in comparison to the Baoule. However, genomic regions affected by divergent selection and environmental adaptation of the Lagune and the Somba remain unknown.

The Lagune cattle from Benin had been exported during the early 20th century (around 1904) to different African countries such as Zaire (current Democratic Republic of Congo), Zambia, Gabon, as well as to Europe where they are known as Dahomey cattle (Rege et al., 1994; Porter et al., 2016), because Dahomey is the former name of the country of Benin. The European Dahomey cattle are presently kept and bred by a breeder association involving 77 farmers (Verband Europäisches Dahomey-Zwergrind, 2019) from four European countries including Germany, Austria, Czech Republic, and Switzerland (http://www.dahomey-zwergrind.com). To date, neither scientific study nor census addressed these animals. According to the breeder association, the current population is characterized by a small size (adult body weight between 150 and 300 kg and sacrum height from 80 to 105 cm), short horn, easy calving, and good temperament. These characteristics are similar to those of the original Lagune population in Benin and may indicate that the Dahomey population conserved its purity through the past decades. However, the European climate is different from the one encountered in Benin, i.e., less heat stress, but varying temperatures between winter and summer, and also varying sunlight duration. In addition, the new production environment of the Dahomey cattle implies the reduction of disease infections with potentially improved feeding and housing systems in opposition to the harsh production conditions in Benin (characterized by food and water scarcity and the risk for disease infections). Consequently, this geographical isolation may have altered frequencies for alleles and haplotypes associated with specific genetic features in the Dahomey population.

The livestock production systems in Benin like in the other African countries have experienced a drastic transformation in the last decades (Mwai et al., 2015; Houessou et al., 2019a). New challenges arise in African pastoral regions along with the increasing demand for animal products, food insecurity, and poverty. Indeed, demographic explosion increases the pressures on animal product markets in West Africa and accentuates the need to develop more productive breeds. Simultaneously, anthropogenic activities such as deforestation and urbanization associated with climate changes have shrunk feed and water resources and increased disease challenges, forcing herders to develop new breeding strategies such as herd mobility or feed supplementation (Houessou et al., 2019a; Ahozonlin and Dossa, 2020). In this context, locally adapted animals are required to cope with the various instabilities in production environments, but the indigenous Shorthorn cattle in Benin are increasingly threatened. The trypanotolerant taurine cattle (without any Zebu ancestry) reported in the region by MacHugh et al. (1997) and Hanotte et al. (2002) are progressively replaced by crossbreeds and trypanosusceptible Zebu cattle, including White Fulani, Sokoto Gudali, and Red Bororo (Dossa and Vanvanhossou, 2016; Houessou et al., 2019b; Ahozonlin and Dossa, 2020). In consequence, significant adoption of prophylactic measures is observed in Beninese herds dominated by crossbreed and Zebu cattle in comparison to other local herds (Houessou et al., 2020).

In regard to the increasing uncontrolled crossbreeding, two other indigenous hybrid cattle, the Borgou and Pabli, are also endangered in Benin. The Pabli originally reported in the region of Kerou (Northwest Benin) is scarcely described and is considered as extinct by absorption from crossbreeding with Borgou (Belemsaga et al., 2005; Egito et al., 2007). However, recent evaluations revealed the existence of a population with slight genetic differences in comparison to the Borgou (Scheper et al., 2020). The Borgou cattle mainly located in the Nord-Eastern and Central regions of Benin were described as an intermediate crossbreed between taurine and indicine cattle (Porter, 1991). The origin of Borgou is still under discussion, but it is assumed to be a product of an admixture between the taurine Somba and the White Fulani Zebu (Porter, 1991; Belemsaga et al., 2005). Flori et al. (2014) characterized the admixture in Borgou as an efficient short-term adaptation strategy to environmental conditions and disease pressures and identified different genomic regions involved in adaptive mechanisms. However, the Borgou cattle population is now highly affected by Zebu cattle influence due to admixture (Scheper et al., 2020). The increased crossbreeding with trypanosusceptible Zebu cattle over a short period questions adaptive features such as resistance to diseases in Borgou, Somba, and Lagune taurine populations. It is therefore urgent to gain more insight into the genetic composition of the current Beninese cattle population to ensure the sustainability of livestock production.

To understand the genetic architecture underlying adaptive and productive abilities of various breeds evolving in challenging environments, selection signature analyses have the potential to detect specific genomic footprints in terms of differences in marker allele frequencies or in haplotypic mosaicism (Qanbari and Simianer, 2014; Aliloo et al., 2020; Kim et al., 2020). According to Freedman et al. (2016), admixture and subsequent recombination break down parental haplotypes and expand mosaic regions through the genome. Thus, extensive admixture in local breeds may reduce the signal of strong homozygosity of extended haplotypes involved in adaptive processes. Moreover, the assessment of homozygous-by-descent (HBD) segments or runs of homozygosity (ROH) is valuable to describe a population and investigate demographic histories. HBD are chromosome segments inherited from an ancestor and may be exploited to estimate inbreeding (Leutenegger et al., 2003).

In this study, we aimed to genetically characterize the current endangered cattle breeds in Benin and evaluate the effects of admixture and environmental factors related to late changes in production systems. Specifically, we first assessed the admixture level in the different populations and compared them with historical samples. Second, through selection signature analysis, we investigated genomic regions and biological mechanisms involved or affected by recent natural or artificial selection and admixture in the Beninese cattle breeds. Subsequently, we investigated the genetic differentiation resulting from the isolation of the Dahomey cattle.

MATERIALS AND METHODS

Sampling Design and Genotype Data

Hair samples were collected from 449 animals from the four local breeds (Borgou 181, Pabli-Kerou 58, Lagune 150, and Somba 60) in Beninese local farms in 2016 and 2017. The sampling locations were identified ensuring a large coverage of the main geographical distribution of the breeds in the country (**Figure 1**). In each herd, one animal representing a (pure) local breed was identified by the farmer and selected. To reduce the relatedness between the samples, one or two animals per village were sampled in 90% of the 298 investigated villages. In the remaining villages, additional animals (three to ten animals in total per village) were sampled for the assessment of further socioeconomic and ecological factors including transhumance and climate (see **Supplementary Table S1A** and Scheper et al. (2020) for more details). Furthermore, thirty Dahomey cattle were sampled in 2019 in 30 different herds across Europe (Germany 23, Austria 4, Switzerland 2, and Czech Republic 1).

The sampled animals were genotyped with the Illumina BovineSNP50 BeadChip. SNP variants with a call rate <95% and a minor allele frequency (MAF < 0.01) as well as animals with a high percentage of missing genotypes (call rate < 90%) were filtered out in PLINK (Purcell et al., 2007). The final genotype dataset including 40,109 SNP from 460 cattle was used for different analyses.

Description of the Current Beninese Cattle Population

Population Structure

The genetic structure of our samples was first assessed by applying the fastStructure software (Raj et al., 2014). The algorithm was run using the simple prior model and testing different numbers of populations (K) ranging from 2 to 10. Afterward, the optimal K was defined using the chooseK.py function. The identified optimal K was finally incorporated into the logistic prior model, to estimate individual genetic proportions describing the population structure, following the recommendations by Raj et al. (2014).

Second, we applied the unsupervised k-means clustering in the "adegenet" R package (Jombart, 2008; Jombart and Ahmed, 2011) to define homogeneous genetic clusters and to exclude potential outgroups animals from the breeds due to sampling. The find.clusters function was applied, considering the following parameters: maximum number of clusters (max.n.clust) = 100, number of principal components (n.pca) = 100, number of iterations in each run (n.iter) = 10^9, number of starting centroids in each run (n.start) = 30, and the default parameters for the remaining arguments (see package description for further details, Jombart, 2008; Jombart and Ahmed, 2011). Finally, the optimal number of genetic clusters was defined by choosing the k-value based on the Bayesian information criterion (BIC), as recommended by the authors (Jombart 2008; Jombart and Ahmed 2011).

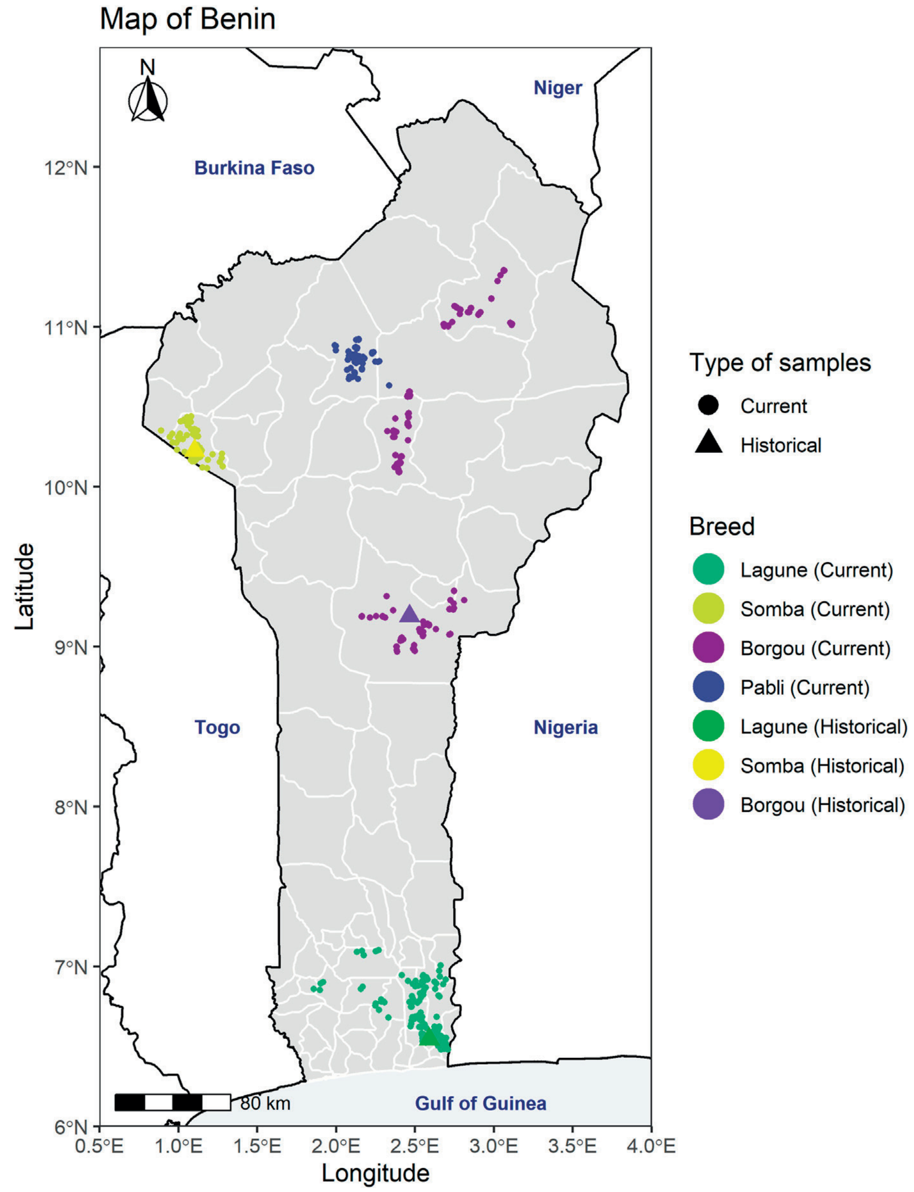

FIGURE 1 | Map of Benin with the sampling locations of the different Beninese cattle populations investigated in the country. For the historical data, the geographical coordinates of each sample are not available. In consequence, the coordinates of the sampling regions as described by Moazami Goudarzi et al. (2001) were plotted: Porto-Novo for the Lagune, Boukombé for the Somba, and the department of Borgou for the Borgou samples.

Genome-Wide Inbreeding Coefficients

We utilized the “RzooRoH” package (Druet and Gautier, 2017; Bertrand et al., 2019) in R to identify HBD and infer the contribution of ancestors from different past generations to inbreeding in our current cattle populations. The software applies a hidden Markov model (HMM) to fit the individual genome as a succession of HBD and non-HBD segments, considering marker allele frequencies, genotyping error rates, and intermarker genetic distances (Druet et al., 2020). In a multiple HBD class model, HBD segments are assigned to K age-related classes associated with different rates (R_k). The class rates (R_k) are related to an expected length and exponential distribution of HBD segments. They are equivalent to twice the number of generations to the common ancestor. The proportion of the genome belonging to a specific HBD class is defined as “realized autozygosity” in the respective HBD class. Similarly, the genome-wide inbreeding coefficient is estimated as the cumulative fraction of the genome that is HBD in the current population with respect to an ancestral base population. As suggested by the authors of the “RzooRoH” package (Druet and Gautier, 2017; Bertrand et al., 2019), we applied a default “MixKR” model with 10 classes (9 HBD classes and 1 non-HBD class) and predefined rates (R_k) 2, 4, 8, 16, 32, 64, 128, 256, 512, and 512, respectively.

Comparison of Current Beninese Cattle Population With Historical Data

Extension of the Genotype Dataset With Historical Data

We contrast the genotype of the current Beninese cattle population with available historical genotypes, retrieved from the WIDDE database (Sempéré et al., 2015). In a first step, the genotype data of 133 animals from Beninese cattle breeds (Borgou 45, Lagune 44, and Somba 44) sampled in different locations (see **Figure 1** and **Supplementary Table S1B**) between 1997 and 2000 (Moazami Goudarzi et al., 2001; Gautier et al., 2009) were merged to the genotype data of our new samples. The dataset with 42,802 SNP from 588 animals was submitted to quality control in PLINK (Purcell et al., 2007), with the parameters --geno 0.05, --mind 0.25, and --maf 0.01. After quality control, the final genotype dataset including 32,533 SNP from 586 cattle was used to apply principal component analysis and unsupervised k-means clustering. Second, further African Shorthorn taurine breed (Baoulé), African Longhorn taurine breed (N'Dama), African indicine breeds (Zebu White Fulani and Zebu Bororo), Asian indicine breeds (Gir, Brahman, Ongole, and Nellore), EUT breeds (Angus, Holstein, Charolais, Shorthorn, and Salers), and African hybrid (Kuri) were included. This extension resulted in 52,341 SNP from 997 animals. However, only 30,637 SNP from 997 animals passed the quality control procedure (with the parameters --geno 0.05, --mind 0.25, and --maf 0.01 in PLINK) and were considered in the principal component analysis, unsupervised k-means clustering, and admixture tests (see below for more details). Finally, 21 Gayal (*Bos frontalis*, Gao et al., 2017) samples were added to the previously extended dataset for the estimation of admixture proportion through the calculation of the f_4-ratio. Likewise, we used the same filtering parameters (--geno 0.05, --mind 0.25, and --maf 0.01) in PLINK. The genotype data consisted of 52,364 SNP and 30,228 SNP from 1,018 animals before and after quality control, respectively. All the genotype data exploited in this study are fully described and available in a public repository (see the section "*Data Availability Statement*"). Before being merged with our samples, the historical genotype data were remapped with the current reference assembly ARS-UCD1.2/bosTau9 (GenBank Bioproject PRJNA391427) and flipped (with the --flip flag in PLINK) to correct for strand inconsistency (Purcell et al., 2007).

Principal Component Analyses and Clustering

The genetic divergence between the Beninese cattle populations and other AFT was assessed using the principal component analysis (PCA) in PLINK (Purcell et al., 2007) and using the unsupervised k-means clustering in "adegenet" (Jombart, 2008; Jombart and Ahmed, 2011) in R. The analyses were subsequently repeated considering EUT and indicine breeds in order to investigate potential introgression of these breeds in our samples, especially in Dahomey. The optimal number of genetic clusters for the unsupervised k-means clustering was defined using the same approach as described above.

Admixture and Estimation of Ancestral Proportion

We tested admixture and inferred admixture proportion in the current and historical Beninese indigenous cattle populations by means of the three-population test (F_3) and the F_4-ratio estimation in Admixtools, respectively (Patterson et al., 2012). The methods are based on f-statistics, corresponding to the average of F values over markers. The Admixtools software uses allele frequencies of the available samples to estimate unbiased f_3 and f_4 statistics (see Patterson et al., 2012, for more details). The three-population test is a formal test of admixture. Negative f_3 (X; B, C) indicates that the allele frequencies in population X tend to be intermediate between B and C and indicates admixture in X populations from populations related to B and C. The f_4 statistics were used to infer admixture proportions in the Beninese cattle populations (target populations) through the calculation of the F_4-ratio or alpha (**Eq. 3**; Patterson et al., 2012). We considered the phylogeny model introduced by Flori et al. (2014) to estimate alpha, as AFT ancestral proportions in our target populations.

$$\text{Alpha} = \frac{f_4\,(A, O; B, C)}{f_4\,(A, O; X, C)}, \tag{3}$$

where X is the target population, A is the Salers population as EUT ancestral, O is the Gayal as outgroup population, B is Baoulé (BAO) as the Shorthorn AFT reference population, and C is Gir as the indicine reference population.

Detection of Regions Under Recent Selection—Gene Annotation and QTL Mapping

We investigated divergence in extended haplotype homozygosity between the current and historical Beninese cattle populations, in order to detect positive selection signatures or genomic footprints left by recent demographic events. Specifically, we compared DAH_cur, LA_cur, and LA_out with LA_hist; SO_cur with SO_hist; and BO_cur and Adm_cur with BO_hist. The cross-population–extended haplotype homozygosity (XP-EHH) approach (Sabeti et al., 2002) was implemented in the "Rehh" package in R (version 3.1.2; Gautier and Vitalis, 2012; Gautier et al., 2017). Prior to the analyses, the main genotype was phased and missing variants were imputed in Beagle 5.1 (Browning et al., 2018). Afterward, integrated site-specific extended haplotype homozygosity (iES) was calculated for each focal marker in the respective population with the "Rehh" package. The XP-EHH statistics were computed as the standardized log ratio of the iES of the two populations. One-sided *p*-values were estimated for XP-EHH to identify strong extended homozygosity in our current populations relative to the respective historical populations (as described above). The estimated *p*-values were subsequently adjusted (Benjamini and Hochberg, 1995) in R. Variants with adjusted *p*-values ≤ 0.05 were considered as significant. In addition, we used a conservative approach similar to those described in previous studies (Flori et al., 2014; Bertolini et al., 2020; Han et al., 2020) and defined as candidate regions under selection, sliding windows of 0.5 MB spanning at least three significant markers. The calc_candidate_regions function in "Rehh" was used to detect the candidate regions. Neighboring

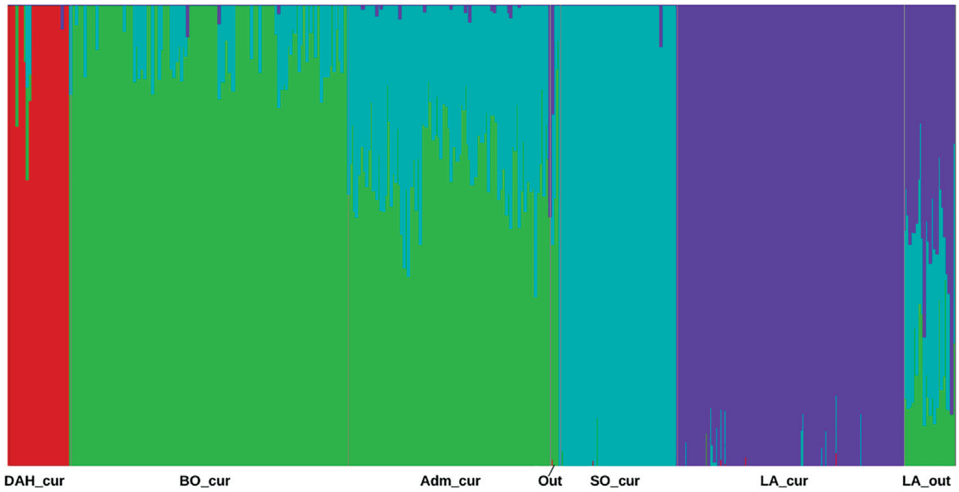

FIGURE 2 | Barplot for admixture proportions inferred by fastStructure in the Beninese cattle populations. Dah_cur = current Dahomey; LA_cur = current pure Lagune; LA_out = admixed Lagune; SO_cur = current Somba; BO_cur = current Borgou highly admixed; Adm_cur = current moderately admixed animal including Pabli with some Borgou, Out = Outgroups (see **Table 1** for more information).

windows with significant SNP were merged to one candidate region. The peak of each candidate region, i.e., the SNP with the lowest adjusted *p*-values in a region, was considered as core SNP.

Genes located in the candidate regions for positive selection signatures were annotated from the Ensembl genome database (2020) (http://www.ensembl.org/biomart/martview/) and submitted to gene ontology (GO) enrichment analysis using the Gene Ontology web-tool (Ashburner et al., 2000; Gene Ontology Consortium, 2019). Fisher's exact threshold of *p*-values < 0.01 was considered to identify overrepresented GO terms for biological processes and Reactome pathways. In addition, QTL that overlapped with the candidate regions under selection were mapped from the online data analysis tools of the cattle database (Hu et al., 2019) and summarized in major production and functional categories: milk, carcass quality, reproduction, body weight, conformation, feed intake, heat tolerance, and health traits (see **Supplementary Table S2** for more details). Subsequently, for each population, we calculated the frequency of the QTL which is equal to the number of candidate regions overlapping with the given QTL per the total number of candidate regions.

RESULTS

Population Structure and Inbreeding in the Current Beninese Cattle Population

Structure of the Current Population

The analyses with the fastStructure algorithm revealed four model components to explain the population structure of our samples. Similarly, the model complexity that maximizes marginal likelihood was equal to four. Considering the posterior mean of admixture proportion in the logistic model, we identified three components representing Somba, Lagune, and Dahomey samples, respectively (**Supplementary Table S3**). The fourth component was mainly made based on Borgou animals. However, some hybrid samples displayed genetic proportions across two or more components. This structure was similar to those obtained with adegenet (**Figure 2**). The unsupervised k-means clustering presented an optimal K (number of clusters, **Supplementary Figure S1**) equal to 6 and displayed the hybrid animals in different three clusters (**Supplementary Figure S1** and **Table 1**). Consequently, a large Lagune group named LA_cur (*n* = 110) was separated from other Lagune samples called LA_out (*n* = 25). Forty-two Borgou and 56 Pabli animals (except two outgroups) formed a homogeneous cluster named Adm_cur (*n* = 98). The remaining Borgou animals were allocated to the BO_cur cluster. The Dahomey and the Somba correspond to the DAH_cur and SO_cur, respectively (**Table 1**). LA_cur, LA_out, DAH_cur, SO_cur, BO_cur, and Adm_cur were considered as populations for further analyses instead of original breed assignments.

Genomic Inbreeding Coefficients

The genome-wide contributions and genomic inbreeding coefficients with respect to different age-related HBD classes in the Beninese cattle populations are presented in **Figures 3A,B**, respectively. Overall, 40% of the genome of DAH_cur samples was HBD and was related only to HBD classes with $R_k \leq 16$. We observed a major contribution of autozygosity from the HBD class with $R_k = 8$, accounting for 63.32% of the total HBD proportion in DAH_cur. The HBD classes with $R_k = 4$ and $R_k = 16$ contributed to 15.70 and 16.80% of the overall autozygosity, respectively. The estimated genomic inbreeding coefficients were 0.17 and 0.13 in LA_cur and LA_out, respectively, when considering all HBD classes (i.e., the most remote base population). The major contribution of autozygosity in LA_cur came from the ancient HBD class with $R_k = 128$ (40% of the HBD proportion). Recent HBD classes with $R_k = 4$ and $R_k = 8$ explained 13.78 and 7.80% of the total autozygosity in LA_cur, respectively, whereas 27.67 and 26.11% of the overall autozygosity in LA_out were derived from the two classes. Tracing back to the oldest ancestors, the fraction of the genome that was HBD in SO_cur was equal to 0.08 and was mainly originated from very ancient HBD

TABLE 1 | Genetic clusters generated from unsupervised k-means clustering applied on the current Beninese cattle population.

Generated genetic clusters		Animals	Breed name assigned by the sampling				
			Borgou	Dahomey	Lagune	Pabli	Somba
Lagune_current	LA_cur	110			110		
Lagune_outgroup	LA_out	25			25		
Dahomey_ current	DAH_cur	30		30			
Somba_ current	SO_cur	57					57
Borgou_ current	BO_cur	135	135			2[a]	
Undescribed_admix	Adm_cur	98	42		2[a]	56	1[a]
Total		455	177	30	137	58	58

[a] *Five animals that did not show clear adherence to the main groups were considered as outgroups and excluded from the generated genetic clusters.*

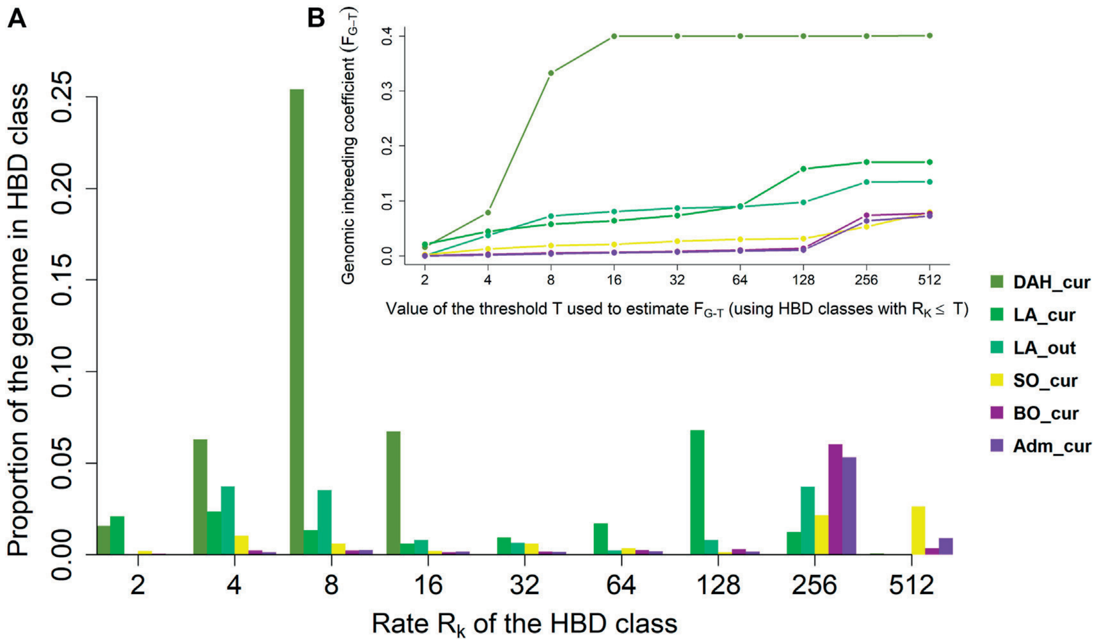

FIGURE 3 | (A) Average realized autozygosity and **(B)** cumulative genomic inbreeding associated with different age-related homozygous-by-descent (HBD) classes in Beninese cattle populations. Dah_cur = current Dahomey; LA_cur = current pure Lagune; LA_out = admixed Lagune; SO_cur = current Somba; BO_cur = current Borgou highly admixed; Adm_cur = current moderately admixed animal including Pabli with some Borgou.

classes with R_k = 256 (27% of the total HBD proportion) and R_k = 512 (33% of the total HBD proportion). The contribution of recent classes to autozygosity in SO_cur was lower (≤13% of the HBD loci). The estimated genomic inbreeding coefficients were 0.08 for BO_cur and 0.07 for Adm_cur. The HBD class with R_k = 256 was the main source of autozygosity, contributing to 77.94 and 73.09% of the total HBD proportions in BO_cur and Adm_cur, respectively.

Comparison of Current Beninese Cattle Population With Historical Data

Principal Component Analyses and Clustering

The first and second principal components (PC) from PCA presented a clear separation of the Beninese breeds (**Figure 4A**). Within each breed, recent and historical samples were distinctly displayed along the second PC. The Dahomey cattle (DAH_cur) were projected next to LA_cur on PC1 but have a major contribution to PC2. Similar differentiations between current and historical samples were observed with the unsupervised k-means clustering, especially in Borgou and Lagune (BIC values suggested seven clusters, see **Supplementary Figure S2A**). Indeed, the unsupervised k-means clustering assigned the animals from Adm_cur, BO_cur, and BO_hist in three different clusters (clusters 2, 6, and 7), respectively (**Supplementary Table S4A**). Similarly, the current Lagune samples (LA_cur and LA_out) were grouped into cluster 4 and historical samples (LA_hist) into cluster 1. DAH_cur formed its own cluster (cluster 4). However, the current and historical Somba samples (SO_cur and SO_hist) were jointly grouped into cluster 5.

We repeated the diversity analyses on the extended dataset including additional AFT, EUT, AFZ, Asian indicine (AID), and

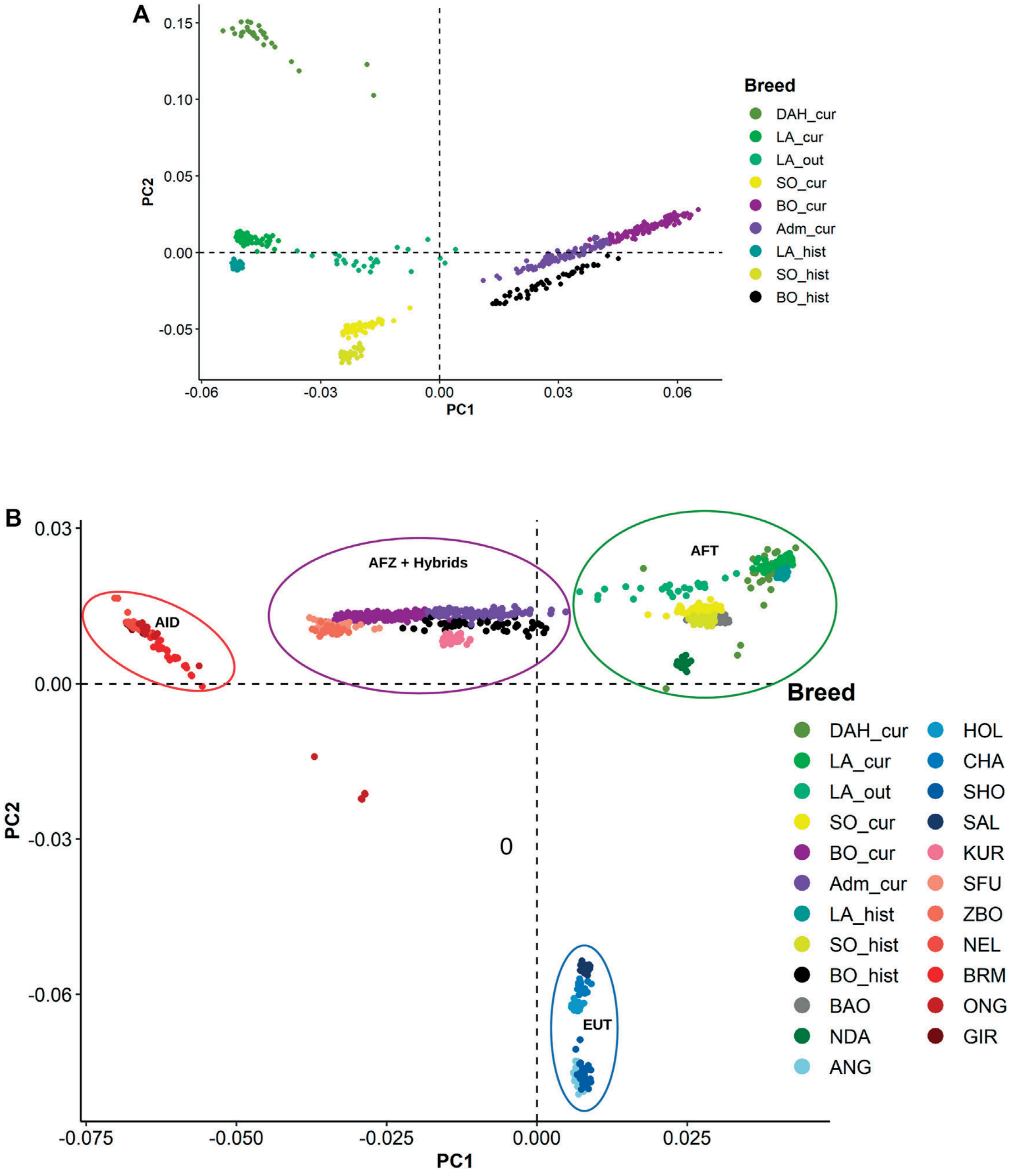

FIGURE 4 | Principal components (PC) displaying genomic divergence: **(A)** in the different Beninese cattle populations; **(B)** in the Beninese cattle with additional African and European taurine (EUT) breeds, African and Asian indicine breeds, and African crossbreeds. Dah_cur = current Dahomey; LA_cur = current pure Lagune; LA_out = admixed Lagune; SO_cur = current Somba; BO_cur = current Borgou highly admixed; Adm_cur = current moderately admixed animal including Pabli with some Borgou; LA_hist = historical Lagune; SO_hist = historical Somba; BO_hist = historical Borgou; ANG = Angus; HOL = Holstein; CHA = Charolais; SHO = Shorthorn; BAO = Baoulé; NDA = N'Dama; GIR = Gir; SFU = Sudanese Fulani; ZBO = Zebu Bororo; BRM = Brahman; ONG = Ongole; NEL = Nellore; KUR = Kuri; SAL = Salers.

hybrids. Considering the PCA, the taurine animals (AFT and EUT) were separated from indicine (AFZ and AID) and hybrids samples along the first component, whereas the second component (PC2) showed a separation between the AFT and EUT (**Figure 4B**). Interestingly, the Dahomey was aggregated with the Lagune far away from EUT. The AID (Gir, Brahman, Ongole, and Nellore) also were clearly separated from the AFZ (ZFU and ZBO) and hybrids. Eight clusters (low BIC at k = 8; **Supplementary Figure S2B**) were identified from the unsupervised k-means clustering. Current and historical Somba samples (SO_cur and SO_hist) formed one genetic group (cluster 2) with the other AFT (BAO and NDA). The

TABLE 2 | f_3 and f_4 statistics for formal test of admixture in Beninese cattle populations.

Target[a]	f_3-ratio	z-score	Alpha[b]
DAH_cur	0.376	26.927	0.974
LA_cur	0.111	35.612	1.000
LA_out	−0.021	−9.677	0.872
SO_cur	0.005	2.492	0.963
BO_cur	−0.083	−39.083	0.474
Adm_cur	−0.048	−28.460	0.615
LA_hist	0.110	35.501	1.008
SO_hist	−0.006	−4.542	0.972
BO_hist	−0.098	−55.732	0.626

[a] *Dah_cur = current Dahomey; LA_cur = current pure Lagune; LA_out = admixed Lagune; SO_cur = current Somba; BO_cur = current Borgou highly admixed; Adm_cur = current moderately admixed animal including Pabli with some Borgou; LA_hist = historical Lagune; SO_hist = historical Somba; BO_hist = historical Borgou.*

[b] *Alpha values represent the estimates of the proportion of AFT in the different populations.*

Lagune samples (LA_cur, LA_out, and LA_hist) were grouped into cluster 2. Similarly, DAH_cur is displayed in one cluster (cluster 1). Moreover, the hybrids (Adm_cur, BO_cur, and BO_hist) were grouped with Kuri, ZFU, and ZBO in cluster 6. The other genetic groups consisted of the EUT and AID (**Supplementary Table S4B**).

Formal Test of Admixture and Inference of Ancestral Proportion

The formal test of admixture of three populations resulted in positive f_3-ratios for DAH_cur, LA_cur, and SO_cur (**Table 2**). In contrast, we obtained negative statistics for LA_out, BO_cur and Adm_cur with the most significantly negative f_3 value for BO_cur ($f_3 = -0.08$; $Z = -39.08$). Considering the historical data, the f_3-ratio test resulted in positive values for LA_hist but was negative for SO_hist and BO_hist.

The estimation of the ancestral AFT with the f_4-ratio test revealed high proportions (alpha values superior to 0.97) in DAH_cur, LA_cur, and SO_cur, respectively (**Table 2**). In contrast, lower alpha values were found for LA_out, Adm_cur, and BO_cur. In comparison to the historical samples, we observed slight reductions of AFT ancestral proportions in Somba (0.96 for SO_cur against 0.97 for SO_hist) and in Adm_cur (0.62 against 0.63 for BO_hist). The current Borgou (BO_cur) displayed an important reduction of AFT ancestral proportions (alpha = 0.47) in comparison to historical samples (BO_hist). For LA_cur and LA_hist, the estimated AFT ancestral proportions were equal to 1.00, respectively.

Selection Signatures

Dahomey and Lagune

We detected no significant SNP presenting strong extended homozygosity in DAH_cur and LA_out relative to LA_hist. In contrast, the current Lagune population (LA_cur) displayed 19 significant SNP for positive selection (**Figure 5A** and **Table 3**). Among the SNP, 12 were positioned in a total of three candidate regions on BTA1, 18, and 21. The region 15.5–16 Mb on BTA21 contained the largest number of significant SNP (six SNP). The region 47.5–48 Mb on BTA18 included the most significant SNP rs110495745 ($p\_adjust = 8.40 \times 10^{-04}$), which is positioned within the *WDR87* gene. In total, 9 candidate genes were annotated within the three regions. GO enrichment analysis identified functional enriched terms such as cellular processes (**Table 4**). QTL associated with reproduction (67%) and conformation (67%) were predominant in the candidate regions (**Figure 6**).

Somba

The analysis of positive selection signatures in SO_cur relative to SO_hist detected 35 significant SNP (**Figure 5B**). Five candidate regions under recent selection in SO_cur were identified on BTA6, 7, 9, and 23, harboring in total 20 significant SNP. The region 4–4.5 Mb on BTA6 displayed the majority of the significant SNP (6 SNP) and the most significant SNP (rs42405104, $p\_adjust = 2.34 \times 10^{-07}$). This SNP is not positioned in any gene. However, the second most significant SNP ($p\_adjust = 3.57 \times 10^{-04}$) is positioned in the vicinity of three genes (*BOLA-DMB*, *BOLA-DMA* and *BRD2*) on BTA 23. In total, 33 genes were mapped in the candidate regions. GO enrichment analysis of the genes revealed highly significant GO biological processes such as antigen processing and adaptive immune response (**Table 4**). We observed that the majority of the candidate regions under positive selection in SO_cur overlapped with QTL affecting heath traits (80%) and carcass quality (60%, **Figure 6**).

Crossbreeds

We detected several genomic regions under recent selection in BO_cur and Adm_cur relative to BO_hist (**Figures 5C,D**). A total of 77 SNP displayed strong homozygosity in BO_cur, with adjusted *p_values* for XP-EHH below the significance threshold ($p\_adjust \leq 0.05$). Among these SNP, 44 were located in eight candidate regions on BTA2, 3, 5, 16, and 27 (**Table 3**). The regions that spanned from 70 to 71 Mb on BTA2 and from 54.5 to 55.5 Mb on BTA5 contained the largest number of significant SNP (9 and 8 significant SNP, respectively). The later region hosted the highest peak with the SNP rs41637710 ($p\_adjust = 0.003$). This SNP was not in the vicinity of any gene. However, three significant SNP (rs110903828 on BTA27, rs41637109 on BTA16, and rs43361717 on BTA3) were positioned within the genes *HTRA4*, *DNAJC11*, and *TMEM53*, respectively. A total of 49 genes were mapped within the genomic candidate regions. GO enrichment analysis revealed several significant GO biological processes including cytoplasmic microtubule organization and inner mitochondrial membrane organization (**Table 4**). Moreover, several QTL affecting animal performances overlapped with the regions under positive selection in BO_cur. QTL associated with milk (75%), carcass (63%), and reproduction traits (63%) were the most represented (**Figure 6**).

In Adm_cur, we identified 69 significant SNP under positive selection. A subset of 42 SNP was located in six candidate regions on BTA3, 4, 5, and 13. Three candidate regions in Adm_cur overlapped with those detected in BO_cur (**Table 3**). Similarly, the SNP rs41637710 located on BTA5 (54.5–55.5 Mb) showed the lowest adjusted *p*-value ($p\_adjust = 3.38 \times 10^{-07}$). The segment on BTA3 (101–102 Mb) hosted the largest number of extreme SNP (13 SNP). The candidate regions in Adm_cur included 63 genes.

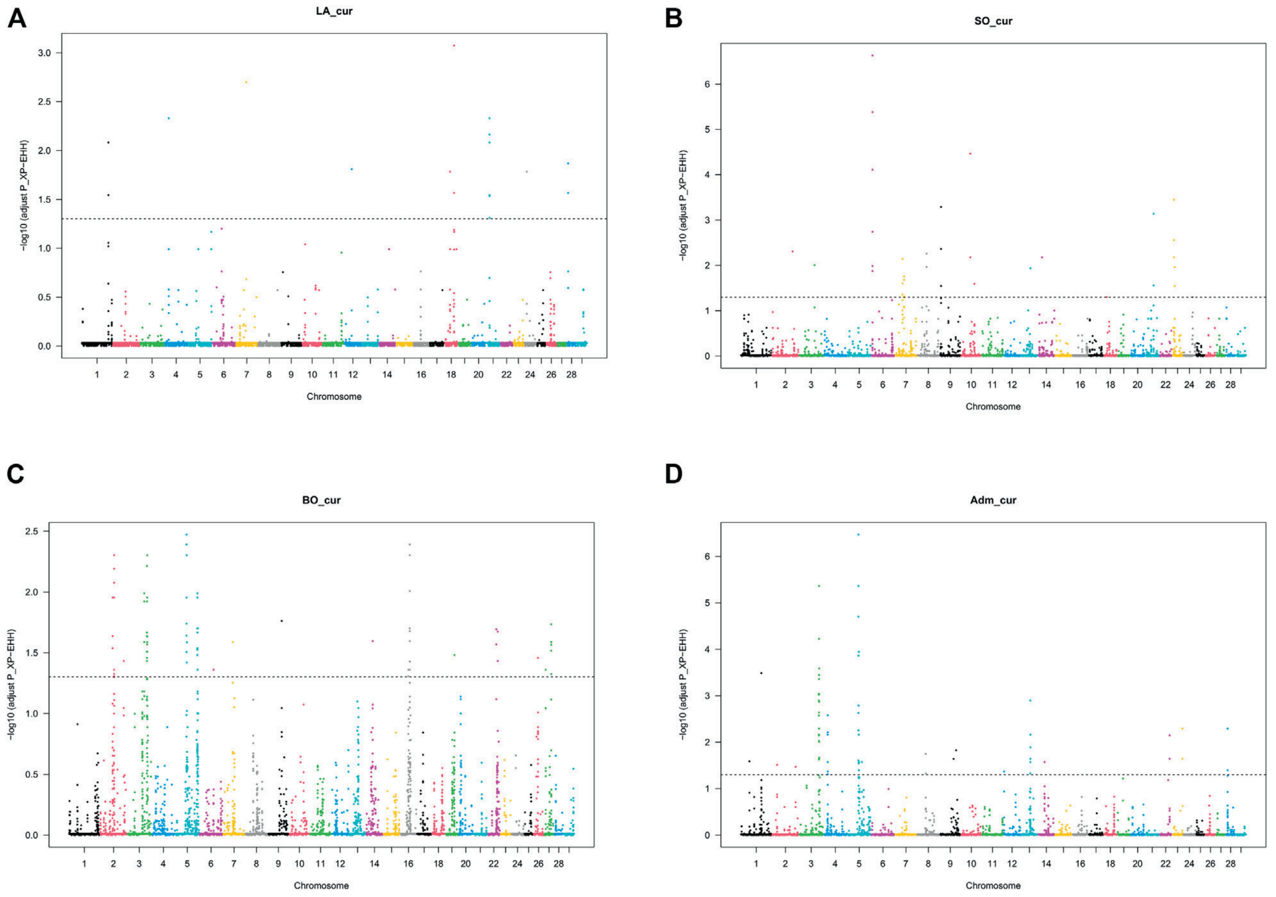

FIGURE 5 | Manhattan plot for cross-population extended haplotype homozygosity (XP-EHH) test for selection signature in Beninese cattle populations: **(A)** LA_cur, current Lagune pure population, **(B)** SO_cur, current Somba population, **(C)** BO_cur, current Borgou highly admixed, and **(D)** Adm_cur, moderately admixed hybrids.

The genes *DNAJC11*, *ENTHD1*, and *MAST2* hosted significant SNP, whereas others (*IL23A*, *PAN2*, and *CNPY2*) were mapped at a close distance to the significant SNP. GO enriched terms included biological processes such as pigment biosynthetic process and response to chemicals (**Table 4**). Like in BO_cur, the majority of QTL, located in the candidate regions of positive selection in Adm_cur, were related to milk (100%), carcass (67%), and reproduction traits (67%, **Figure 6**).

DISCUSSION

Population Structure and Admixture Tests

The results of the population structure analyses are in line with the breed foundation of the different cattle populations in Benin and their divergence from other African and European cattle breeds (Mwai et al., 2015). First, we observed high proximity of the Somba and Baoulé populations as Savannah Shorthorns and their separation to the Dwarf (forest) Lagune Shorthorn (Rege et al., 1994). In addition, the divergence of the Shorthorn breeds from the Longhorn N'Dama illustrates the rich genetic diversity of West African indigenous cattle breeds and the necessity to unravel specific signatures in each population. Second, our study confirms the Lagune origin of the Dahomey cattle. We found neither indicine nor EUT background in the Dahomey population. The genetic purity of the Dahomey cattle is probably due to their promotion in a close production system organized by the Dahomey-Zwergrind breeder association aiming at the conservation of the breed (http://www.dahomey-zwergrind.com). The inference of the oldest common ancestor dated from eight generations ago (approximately 24 years ago). The high genomic inbreeding coefficient in $R_k = 8$ (4 generations equal to 16 years ago) in the Dahomey population may be related to the recent creation of the association in 2001. These results suggest that the founders of the current Dahomey population kept by farmers may have originated from a small number of Dahomey cattle four generations ago (http://www.dahomey-zwergrind.com). However, the low frequency of short ROH segments, resulting in low genomic inbreeding coefficients (<0.1) in very young classes ($R_k \le 4$), indicates a reduction of mating between related individuals in recent generations (Druet and Gautier, 2017). Exchange of breeding animals between the association members and consideration of Dahomey cattle currently held in different zoos across Germany and other

TABLE 3 | Candidate regions harboring positive recent selection signatures in Beninese cattle populations.

Population[a]	BTA	Region (Mb)		Number of sign. SNP	Adjust p-value		Genes[b]
		Start	End		Min	Max	
LA_cur	1	137.5	138	3	0.008	0.029	
	18	47.5	48	3	0.001	0.027	***WDR87***, *ZNF345, ZFP30*
	21	15.5	16	6	0.005	0.049	*SV2B*
SO_cur	6	4	4.5	6	0.000	0.013	QRFPR
	7	45	45.5	5	0.018	0.049	*FSTL4*
	9	4	4.5	3	0.001	0.028	
	23	7	7.5	3	0.000	0.007	***BOLA-DMA***, ***BOLA-DMB***, ***BRD2***, *PSMB8*
	23	11.5	12	3	0.011	0.028	*MDGA1, ZFAND3*
BO_cur	2	70	71	9	0.005	0.050	EN1, MARCO
	3	86	86.5	3	0.010	0.026	HOOK1
	3	100	100.5	5	0.006	0.035	**MAST2**, RAD54L, POMGNT1
	3	101	101.5	4	0.005	0.027	HECTD3, KIF2C
	5	54.5	55.5	8	0.003	0.031	*LRIG3*
	5	111	111.5	6	0.010	0.033	GRAP2, **ENTHD1**, FAM83F
	16	46.5	47	6	0.004	0.025	***DNAJC11****,* RNF207, *PLEKHG5, THAP3*
	27	34	34.5	3	0.026	0.047	*PLEKHA2, ADAM32,*
Adm_cur	3	100	100.5	6	0.001	0.007	*MAST2, RAD54L, POMGNT1*
	3	101	102	13	0.000	0.023	HECTD3, **RNF220**, **IPP**
	4	20	21	6	0.003	0.049	TMEM106B, SCIN, ARL4A
	5	54.5	55.5	8	0.000	0.025	LRIG3
	5	57	57.5	6	0.000	0.040	ANKRD52, SLC39A5, RNF41, DNAJC14
	13	45.5	46	3	0.001	0.047	—

[a] *LA_cur = current pure Lagune; SO_cur = current Somba; BO_cur = current Borgou highly admixed; Adm_cur = current moderately admixed animal including Pabli with some Borgou.*
[b] *Genes harboring the core SNP are displayed in bold. The complete list of the genes located in the candidate regions are presented in* ***Supplementary Table S5****.*

European countries (Zootierliste, 2020: https://www.zootierliste.de) may contribute to control inbreeding and to increase genetic diversity in the population currently managed by the Dahomey–Zwergrind breeder association.

The results of the PCA analyses differentiated historical and current populations as well as populations affected by admixture. The identification of admixed animals (LA_out) from relatively pure Lagune is confirmed by the formal admixture test, the three-population test, and the estimation of admixture proportions. Our findings are in accordance with the increasing crossbreeding in Lagune cattle due to the extension of transhumance as reported by Scheper et al. (2020) and Ahozonlin and Dossa (2020). The large genomic inbreeding in the Lagune (LA_cur) is in line with the small number of the original populations formerly distributed in clusters across West African coastal and forest regions (Rege, 1999). Fortunately, the estimation of high AFT ancestral proportion in LA_cur suggests the existence of a relatively pure population, which may be valuable for the conservation of this indigenous taurine breed. The estimated alpha value of 1.00 may be related to the reference population considered. However, the Baoulé (BAO) is the closest shorthorn taurine with available historical genotype data, whereas the GIR is the purest indicine reference population as the majority of AFZ are admixed (Flori et al., 2014).

We found that Somba cattle are less affected than Lagune by Zebu introgression. Previous studies observed that its habitat in the hilly region of Atacora protected from Zebu introgression (Rege et al., 1994; Hall et al., 1995). In comparison to the location of the other local breeds (Northeastern and Southern Benin), the lower pressure of transhumance in Boukombe and lower economic and demographic pressures, resulting in less "modernization" of cattle management and crossbreeding, are some advantages (Houessou et al., 2019a; Scheper et al., 2020). However, the lower AFT ancestral proportion in SO_cur compared to SO_hist confirms the threat of admixture in Somba cattle mainly caused by entrustment practices (Hall et al., 1995; Dossa and Vanvanhossou, 2016; Vanvanhossou et al., 2021). In addition, the negative and positive f-statistics in the historical (SO_hist) and the current (SO_cur) Somba populations, respectively, indicate former introgression episodes followed by genetic drift (Patterson et al., 2012; Kim et al., 2020). We also observed that Somba cattle remain less inbred despite the reduction of population size and their shrinkage into the unique location of Boukombe (Dossa and Vanvanhossou, 2016).

The results from fastStructure corroborate the Somba background of the Beninese crossbreeds. In addition, the hybrid populations (BO_cur and Adm_cur) presented a relatively low genetic proximity to the AID (Gir, Brahman, Ongole, and Nellore), but they were clustered close to the AFZ (ZFU and ZBO). These results are in agreement with their origin as described by different authors (Atchy, 1976; Flori et al., 2014). The identification of different levels of admixture in the hybrid samples was confirmed by the f_3 and f_4 admixture tests. The lower AFT ancestral proportion in BO_cur compared to the historical population BO_hist confirms the increasing introgression of AFZ in smallholder Borgou herds as indicated by Scheper et al. (2020). BO_cur representing more than 75% of the current Borgou samples suggests an intensive admixture and a high risk of full replacement of the Borgou population by AFZ genotypes. We observed similar AFT ancestral proportions in the historical Borgou (Bo_hist) and Adm_cur. The AFT ancestral

TABLE 4 | Enriched gene ontology (GO) biological process for genes in candidate regions under positive selection in Beninese cattle populations.

Population[a]	GO biological process	p- value	N[b]	Genes
LA_cur	Cellular process (GO:0009987)	5.94E-03	2	*SV2B, ENSBTAG00000054913*
SO_cur	Antigen processing and presentation (GO:0019882)	5.44E-19	11	*BOLA-DOA, BOLA-DMA, TAP1, PSMB8, TAPBP, DSB, BOLA-DMB, BOLA-DYA, BOLA-DOB, BOLA-DMA, BOLA-DIB*
	Adaptive immune response (GO:0002250)	2.74E-11	9	*BOLA-DOA, BOLA-DMA, TAP1, DSB, BOLA-DMB, BOLA-DYA, BOLA-DOB, BOLA-DMA, LOC618733*
	MHC protein complex assembly (GO:0002396)	3.98E-05	2	*BOLA-DMA, TAPBP*
	Cellular response to steroid hormone stimulus (GO: 0071383)	6.61E-03	2	*RXRB, DAXX*
	Proteasomal ubiquitin-independent protein catabolic process (GO:0010499)	7.03E-04	2	*PSMB8, PSMB9*
	DNA conformation change (GO:0071103)	9.03E-03	3	*H2B, BRD2, DAXX*
BO_cur	GO:000700: inner mitochondrial membrane organization (GO:0048519)	2.42E-03	2	*TAZ, DNAJC11*
	Negative regulation of biological process (GO: 0048519)	5.20E-03	2	
	Cytoplasmic microtubule organization (GO: 0031122)	7.61E-03	2	*HOOK1, PLK3*
	Biological regulation (GO:0065007)	6.25E-03	16	*EIF2B3, KLHL21, ADAM9, KIF2C, TNFRSF25, MAST2, THAP3, PTCH2, ZBTB48, HES2, PLK3, TAS1R1, PLEKHG5, EN1, GPBP1L1, FAM83F*
Adm_cur	Pigment biosynthetic process	7.01E-03	2	*UROD, PMEL*
	ATP-dependent chromatin remodeling	7.90E-03	2	*DMAP1, SMARCC2*
	Response to chemical	9.33E-03	3	*SLC39A5, PLK3, ENSBTAG00000051912 (taste receptor type 2)*
	G1/S transition of mitotic cell cycle	9.50E-03	2	*CDK2, PLK3*

[a] *LA_cur = current pure Lagune; SO_cur = current Somba; BO_cur = current Borgou highly admixed; Adm_cur = current moderately admixed animal including Pabli with some Borgou.*
[b] *Number of the identified genes.*

proportion in the later population, comprising the Pabli samples, indicates the existence of a residue of the Beninese indigenous crossbreeds. According to Pecaud (1912) (as reported by Atchy, 1976), the Pabli breed results from crossbreeding between Borgou and Somba around the year 1905. In addition, the region of Kerou hosting the Pabli cattle in Western Benin is also less affected by transhumance. The association between spatial indicine introgression and transhumance in the Beninese cattle population was described by Scheper et al. (2020). Finally, the existence of crossbreed populations with divergent admixture levels offers the opportunity to evaluate the impact of crossbreeding in terms of divergence in extended haplotype homozygosity profiles.

Selection Signatures

Previous studies (Lohmueller et al., 2010; Freedman et al., 2016) observed that admixture or further demographic events (population bottlenecks due to diseases) affect ancestral haplotypes and increase the occurrence of mosaics in the genome (Freedman et al., 2016; Aliloo et al., 2020). This may impede the distinction of genomic footprints left by neutral processes and natural selection. In consequence, we did not expect evidence for historical selections in the Beninese cattle population. Nevertheless, the selection is not dissociable from admixture in several African cattle breeds. Admixture is a historical practice in African cattle production and is considered as a quick means of animal upgrading (Flori et al., 2014). Researchers reported that the selection of the animal or the breed for crossbreeding is driven by farmer interests including desired productive (milk, meat, and reproductive) and adaptive features (left by natural selection) (Boutrais, 2007). In this context, the prevalence of specific genomic regions or functional traits within a population may reflect the production goals of the farmers. In addition, specific features in the genome of African cattle populations (including several populations with various crossbreeding histories) are commonly assessed with selection signature analyses (Gautier et al., 2009; Taye et al., 2018; Aliloo et al., 2020; Kim et al., 2020).

By contrasting the current and historical cattle populations in the Beninese taurine and crossbreeds, we focus on genomic footprints resulting from recent environmental pressures or herd management. Indeed, environmental and socioeconomic factors have induced diverse changes in cattle management practices in Benin. These include the adoption of cattle mobility in taurine or agropastoralist herds (former sedentary), the increase of herd mobility frequencies and amplitudes, the migration and settlement of several agropastoralists from Sahelian countries or Northern Benin into Southern Benin, and the extension of animal entrustment practices (from agropastoralists to traditional pastoralists) (Houessou et al., 2019a; Houessou et al., 2020; Vanvanhossou et al., 2021). Despite the limited period between our and the historical samples, we identified a few candidate regions, providing new insights into the evolutionary process in the indigenous breeds. In comparison to the taurine, we observed that the crossbreeds, especially BO_cur, displayed most of the candidate regions. These results are in line with increased Zebu introgression in BO_cur, leading to a higher admixture proportion than Adm_cur and more genetic divergence from the historical Borgou (BO_hist). Regarding the Somba (SO_cur), the identification of five candidate regions mainly associated with immunity features suggests the importance of disease pressures in its belt, as extensively discussed below.

Our approach to detect a strong signal of homozygosity considering temporal subpopulations within the same breed is similar to the one applied by Naderi et al. (2020), who contrasted the current German Holstein to one of its recent ancestors, the local dual-purpose German black pied cattle (DSN). In addition, we aimed to reduce the bias due to the uncertainty of the ancestral base population and applied the XP-EHH method to detect complete sweeps in contrast to the Integrated Haplotype Score (IHS) approach (Qanbari and Simianer, 2014). Indeed, the IHS method usually applied to investigate within-breed selection signatures relies on ancestral allele frequency which is inconsistently defined in different studies. For instance, Utsunomiya et al. (2013) derived ancestral alleles from common founders of Bovidae species, namely, *Bos gaurus*, *Bos grunniens*, and *Bubalus bubalis*. Kim et al. (2020) considered fixed alleles in African Buffalo as ancestral alleles to determine selection signatures in the African population, while Gautier et al. (2009) estimated ancestral alleles for West African cattle breeds based on alleles frequencies in indicines and African and European taurine samples. Finally, we expect to reduce false-positive results due to multiple tests, by considering adjusted *p*-values to define significant SNP and by defining only regions with at least three significant SNP as candidate regions. The respective candidate regions identified for each cattle population are discussed in the ongoing sections.

Lagune

We were not able to detect any candidate regions for DAH_cur. The Dahomey cattle may have acquired very few complete selective sweeps that are not detectable by the XP-EHH. This result reflects the breeding strategy with only focus on leisure and is in line with its high genomic inbreeding coefficient (http://www.dahomey-zwergrind.com). Further investigations including a complete sequencing of the Dahomey cattle and other methods of selection signature analyses may help untangle the genetic divergence between the Dahomey and the Lagune.

The admixed LA_out population was not considered for selection signature analysis because of the divergence in introgression levels as shown in the population structure analyses and admixture test. The LA_out group is the product of diverse admixed animals sampled as Lagune and therefore cannot represent any specific cattle population in Benin. On the contrary, LA_cur, as a relatively pure Lagune population, displayed three candidate regions. The region 15.5–16 Mb on BTA21 including the most significant SNP in LA_cur is identical to selection signatures reported in Holstein and North African cattle (Taye et al., 2017b; Ben-Jemaa et al., 2020). The *SV2B* gene in this region is associated with feed intake in cattle (Seabury et al., 2017). In contrast to the other populations investigated in this study, the candidate regions under selection in LA_cur encompassed relatively few genes functionally described in the literature. Nevertheless, the *WDR87* gene, hosting the most significant SNP, is related to carcass traits in cattle (Lim et al., 2013). In addition, the majority of the genes are involved in cellular and regulation of RNA biosynthetic processes, which are responsible for feed efficiency and body weight (Olivieri et al., 2016). Similarly, QTL associated with body weight, milk and reproductive traits are predominant in the candidate regions.

Somba

With regard to the five candidate regions under selection in SO_cur, two were detected on BTA23. This chromosome is

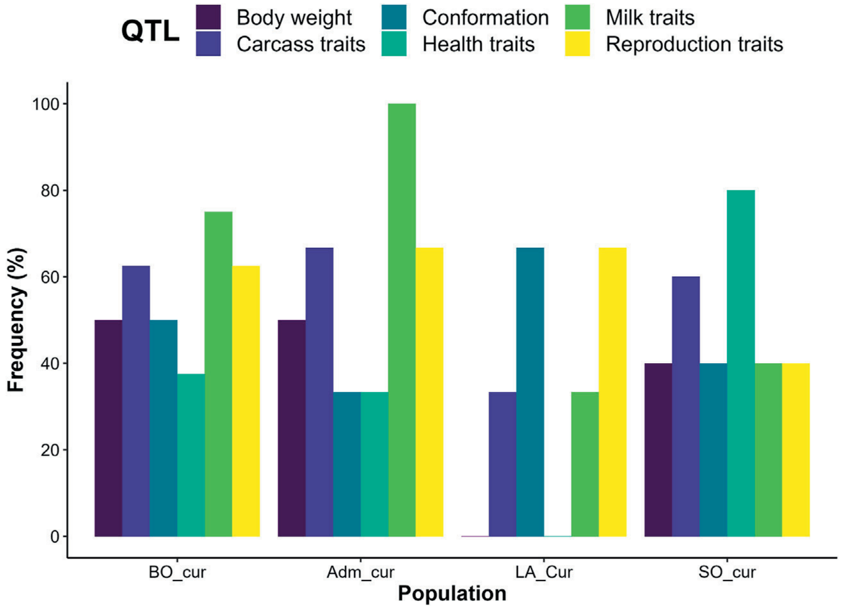

FIGURE 6 | Proportion of candidate regions with quantitative trait loci (QTL) controlling varied trait categories. LA_cur = current pure Lagune; SO_cur = current Somba; BO_cur = current Borgou highly admixed; Adm_cur = current moderately admixed animal including Pabli with some Borgou.

known to have two subregions (classes IIa and IIb) of the bovine leukocyte antigen (BoLA), also called bovine major histocompatibility complex (MHC), which is determinant in the development of acquired immune responses to diverse parasitic and viral diseases (Ellis and Ballingall, 1999; Takeshima and Aida, 2006). The selective region 7–7.5 Mb (BTA23) spanning several genes (e.g., *DSB*, *BOLA-DYA*, *BOLA-DMB*, and *BOLA-DOA*) overlaps with the subregion of BoLA class IIb (Takeshima and Aida, 2006). These BoLA class IIb genes are specific to ruminants but are less characterized, in contrast to genes in the BoLA class IIa (e.g., *BoLA-DRB3* and *BoLA-DQA3*) (Takeshima and Aida, 2006). In addition, Ballingall and McKeever (2005) associated the rare polymorphism of BoLA class IIb genes to evolutionary processes under functional constraints. Selection signatures in this genomic region were only found in Angus and Brangus cattle (Goszczynski et al., 2018; Maiorano et al., 2018), whereas selection for adaptive immunity in African cattle breeds is usually identified within the BoLA class IIa region (Ballingall et al., 1997; Kim et al., 2017; Tijjani et al., 2019). Therefore, recent selection signatures in the specific BoLA class IIb genes may indicate a possible adaptation of the Somba cattle to endemic diseases, especially to anthrax. Indeed, the Somba cattle are affected by several recent episodes of anthrax outbreaks especially in the years 2007, 2009, 2012, and 2013 (Dossa and Vanvanhossou, 2016). Further studies are required to investigate the association between BoLA subregion IIb and resistance to disease in Somba and other African cattle breeds. In addition, other candidate genes include *PSMB9* and *HSD17B8* which are involved in meat and growth traits (Lee et al., 2012; Ma et al., 2015), *FSTL4* associated with milk production (Sanchez et al., 2019) and *ZFAND3* responsible for reproduction (Mohammadi et al., 2020). The predominance of candidate genes associated with immune response is confirmed by enriched terms such as antigen processing and presentation, and adaptive immune response. However, other biological processes include the regulation of cellular metabolic processes and intracellular protein transport mechanisms. The identified bovine QTL suggests selection on body weight, carcass, reproduction, and milk traits in the Somba cattle.

Crossbreeds

The regions of selection (54.5–55.5 Mb on BTA5, 100–100.5 Mb, and 101–101.5 Mb on BTA3) overlapping in the two hybrid populations are in line with their common indicine background. The significant SNP mapped in the region from 100 to 101.5 MB on BTA3 were positioned in various genes including the *RNF220* gene. This gene has been previously identified under selection in West African cattle (Gautier et al., 2009). In addition, the region includes genomic footprints of signatures in South African and East African Shorthorn hybrids with indicine ancestry deficiency in the later breed (Bahbahani et al., 2015; Zwane et al., 2019). These findings suggest that the region may represent an ancient and stable footprint of selection in indigenous African hybrids. The *RNF220* gene is involved in calving performance and milk yield (Abdel-Shafy et al., 2020; Purfield et al., 2020). In addition, the HECTD3 in this region is associated with cell cycle regulation and fat deposition, while PLK3 is related to gain and feed intake in cattle (Yu et al., 2009; Bahbahani et al., 2015; Zarek et al., 2017). We also detected in this region the PTCH2 and *SLC6A9* which are involved in reproduction and Porphyria disease, respectively (Nezamzadeh et al., 2005; Basavaraja et al., 2021). The region 55–55.5 Mb on BTA5 previously displayed evidence of selective sweeps in the EUT Charolais as well as in a tropical crossbreed between Charolais and Zebu, namely, the Canchim (Xu et al., 2015; Urbinati et al., 2016; Naval-Sánchez et al., 2020). Moreover, the *LRIG3* gene in this region is associated with body length in cattle and litter size in pigs (Xu et al., 2015; Metodiev et al., 2018).

Specific selection signatures detected in BO_cur include the selective sweep 46.5–47.5 Mb on BTA16. This region is of great interest. It presents several significant SNP and overlaps with genomic footprints detected in East African Zebu cattle as well as in a subpopulation of the German dual-purpose black and white cattle (Taye et al., 2018; Naderi et al., 2020). The region contains *DNAJC11*, a heat shock protein gene, involved in response to heat stress (Li et al., 2015). In addition, the candidate regions of selection (BTA 5:111.5–111.5 Mb and BTA 2:70–71 Mb) are known candidate regions under selection in different African, European and Asian cattle and sheep breeds (Hudson et al., 2014; Bomba et al., 2015; Wang et al., 2015; Bertolini et al., 2020). The region 111.5–111.5 Mb on BTA5 contributed to positive selection for natural virus resistance and to extensive admixture in West Sahelian African human populations (Cagliani et al., 2011; Pérez-Rivas et al., 2014; Triska et al., 2015). In addition, the segment BTA 2:70–71 Mb covered various candidate genes such as *EN1*, involved in growth traits in cattle (Buroker, 2014). Further regions under selection in BO_cur host candidate genes, significantly associated with different traits, including *ADAM32*, *ADAM9*, *HTRA4*, and *KLHL21* with residual feed intake and immune responses, *PLEKHA2* and *TNFRSF25* with growth and carcass performances, *HTRA4* with milk, and *HOOK1* with heat stress (Fan et al., 2015; Tizioto et al., 2015; Seong et al., 2016; Blanco et al., 2017; Hardie et al., 2017; Hay and Roberts, 2018; Sengar et al., 2018; Skibiel et al., 2018; Oliveira et al., 2019; Braz et al., 2020; Brunes et al., 2021; Soares et al., 2021).

Regarding the selection signatures in Adm_cur, the region 20–21 Mb on BTA4 in Adm_cur is consistent with the region reported by Naderi et al. (2020), who identified selection signatures in close distance to the *TMEM106B* gene in the German black pied cattle. In addition, the *ARL4A* gene was reported in the context of selective sweeps in Australian Holstein (Larkin et al., 2012) and with regard to copy number variations in African Nguni cattle and Polish Holstein (Wang et al., 2015; Mielczarek et al., 2017). The latter gene is associated with milk production in dairy (Raschia et al., 2018; Khan et al., 2020). The *SCIN* gene, also mapped in the region, is involved in residual feed intake in cattle (Salleh et al., 2017). The remaining regions under selection in Adm_cur spanned other candidate genes including *ANKRD52*, *RNF41*, and *MYL6* associated with height and carcass traits (Cai et al., 2019; Moravčíková et al., 2019; Feitosa et al., 2021), and *COQ10A* and *RNF41* related to milk trait and calf mortality (Lázaro et al., 2021; Marín-Garzón et al., 2021).

In addition, the *SARNP* gene is related to animal reproduction (Labrecque et al., 2014), while the *DNAJC14* is involved in heat stress (Bahbahani et al., 2015; Rehman et al., 2020).

Overall, the common and specific candidate regions identified in the hybrid populations confirm selection signatures in African and European crossbreeds (**Supplementary Table S6**). They cover several candidate genes related to economic and functional traits. The enriched biological processes including inner mitochondrial membrane organization and ATP-dependent chromatin remodeling are related to carcass traits, milk production, and reproduction in cattle (Lu et al., 2016; Zhang and Xie, 2019; Shi et al., 2021). We also observed that the hybrids present more candidate regions related to heat response than the taurine populations, which is in line with the admixture with Zebu cattle known to tolerate high environmental heat loads (Taye et al., 2017a; Kim et al., 2020). Moreover, few genes in candidate regions of selection are involved in immune response and feed efficiency. The evidence of selection for adaptive traits in the hybrid populations (including BO_cur that is highly admixed) may be related to the fact that the AFZ introduced in the production environments of the West African taurine for decades have also developed various adaptive features (Atchy, 1976; Houessou et al., 2019b). Consequently, their crossbreeding with indigenous taurine cattle reduces the risk of diluting adaptive traits in local breeds while offering the opportunity to increase animal performances. Our findings are in line with those reported in other African cattle breeds (Aliloo et al., 2020; Kim et al., 2020) and suggest the ability to develop robust and productive breeds via crossbreeding. Nevertheless, the improvement of cattle breeding in West Africa requires the establishment of sustainable crossbreeding programs and the enhancement of genomic selection including genotype by environment interactions in the indigenous breeds. These will be achieved through the determination of suitable breeds and optimal proportions of admixture, considering social-ecological constraints (Wollny, 2003; Wurzinger et al., 2014).

CONCLUSION

In this study, we confirm that the Dahomey cattle currently bred in Europe are a subpopulation of the Beninese indigenous Lagune breed. The high genomic inbreeding in the Dahomey population is due to its current breeding system. The introduction of new animals from zoological parks can increase the diversity of the Dahomey population. Moreover, the Beninese taurine indigenous Lagune and Somba cattle still conserve a high proportion of AFT ancestry, in comparison to the historical population. The Borgou displays a risk of full genetic replacement by African Zebu. However, we observed the existence of a hybrid population relatively less affected by ongoing indicine introgression and comparable to the historical Borgou.

We found no evidence of the negative impact of admixture on the adaptive features in the cattle populations including the crossbreeds, as they all present several genomic footprints involved in immune response, feed efficiency, and heat stress. Moreover, specific candidate regions in the Somba cattle demonstrate selection pressures related to endemic diseases in the habitat areas of the breed. Overall, identified recent selection in Beninese indigenous cattle towards productive traits such as reproduction, milk, and carcass traits favor the improvement of the economic merits of the breeds.

DATA AVAILABILITY STATEMENT

All the data supporting the results of this article are presented within the article or in the additional files. The raw genotypic data used in this study are openly accessible at http://dx.doi.org/10.22029/jlupub-73.

AUTHOR CONTRIBUTIONS

SV, CS, LD, and SK conceived the project. SU collected the data, designed and applied the statistical analyses, and drafted the manuscript. TY and CS supported in statistical analyses and manuscript preparation. SK, RF, and LD reviewed and edited the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2021.657282/full#supplementary-material

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Publication 3. A Multi-Breed GWAS for Morphometric Traits in Four Beninese Indigenous Cattle Breeds Reveals Loci Associated with Conformation, Carcass and Adaptive Traits

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A multi-breed GWAS for morphometric traits in four Beninese indigenous cattle breeds reveals loci associated with conformation, carcass and adaptive traits

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Abstract

Background: Specific adaptive features including disease resistance and growth abilities in harsh environments are attributed to indigenous cattle breeds of Benin, but these breeds are endangered due to crossbreeding. So far, there is a lack of systematic trait recording, being the basis for breed characterizations, and for structured breeding program designs aiming on conservation. Bridging this gap, own phenotyping for morphological traits considered measurements for height at withers (HAW), sacrum height (SH), heart girth (HG), hip width (HW), body length (BL) and ear length (EL), including 449 cattle from the four indigenous Benin breeds Lagune, Somba, Borgou and Pabli. In order to utilize recent genomic tools for breed characterizations and genetic evaluations, phenotypes for novel traits were merged with high-density SNP marker data. Multi-breed genetic parameter estimations and genome-wide association studies (GWAS) for the six morphometric traits were carried out. Continuatively, we aimed on inferring genomic regions and functional loci potentially associated with conformation, carcass and adaptive traits.

Results: SNP-based heritability estimates for the morphometric traits ranged between 0.46 ± 0.14 (HG) and 0.74 ± 0.13 (HW). Phenotypic and genetic correlations ranged from 0.25 ± 0.05 (HW-BL) to 0.89 ± 0.01 (HAW-SH), and from 0.14 ± 0.10 (HW-BL) to 0.85 ± 0.02 (HAW-SH), respectively. Three genome-wide and 25 chromosome-wide significant SNP positioned on different chromosomes were detected, located in very close chromosomal distance (±25 kb) to 15 genes (or located within the genes). The genes *PIK3R6* and *PIK3R1* showed direct functional associations with height and body size. We inferred the potential candidate genes *VEPH1, CNTNAP5, GYPC* for conformation, growth and carcass traits including body weight and body fat deposition. According to their functional annotations, detected potential candidate genes were associated with stress or immune response (genes *PTAFR, PBRM1, ADAM TS12)* and with feed efficiency (genes *MEGF11 SLC16A4, CCDC117*).

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Conclusions: Accurate measurements contributed to large SNP heritabilities for some morphological traits, even for a small mixed-breed sample size. Multi-breed GWAS detected different loci associated with conformation or carcass traits. The identified potential candidate genes for immune response or feed efficiency indicators reflect the evolutionary development and adaptability features of the breeds.

Keywords: Endangered cattle breeds, Morphometric traits, Multi-breed GWAS, SNP-based genetic parameters, Functional annotations, Potential candidate genes

Background

In Sub-Saharan Africa, the cattle breeding sector comprises smallholder subsistence farms in extensive production systems, and only a few exclusively market-oriented productions. In consequence, there is a substantial gap with regard to organized breeding programs and routine performance testing, technical support and precise breeding policies. Against such a background, applying advanced breeding technologies combined with enhanced genomic statistical methods in order to conserve animal genetic resources remains a major challenge, as observed in many developing countries [1, 2]. First approaches to utilize genomic information in animal breeding in Africa were recently made, but based on limited resources [2, 3]. Nevertheless, the African continent is well-endowed with hundreds of indigenous, often endangered, animal genetic resources. In addition to being the source of livelihood for millions of poor farmers, these genetic resources represent specific socio-economic values, and they are unique for traits indicating adaptation to harsh environments [4–8].

High-throughput genotyping and genomic methods provide new opportunities for the genetic characterization and genetic management of African indigenous animal breeds. An elementary genetic and phenotypic evaluation is the prerequisite for future performance improvements. Such efforts might be the key to successful management and conservation of animal genetic resources in the light of policy developments, climate change, and diversifying market demands [9–11]. In this regard, studies addressing selection signatures analyses for heat tolerance, thermoregulation, tick resistance and trypanotolerance in African cattle breeds reflect the unique potential and advantage they possess [12–15]. Therefore, more efforts are needed to investigate the genetic architecture of functional and performance traits in native African breeds, in order to unravel their potential for future breeding development.

A great variety of study designs and methods for GWAS as established in recent years are powerful tools to study the genomic architecture of both qualitative and quantitative traits. Consequently, series of GWAS have been performed for performance and functional traits in various livestock species and breeds in European, American and Asian countries, e.g. [16–19]. In Africa, however, only a few GWAS in livestock have been reported to date [20–22], due to the difficulties in collecting valid phenotypic data in the smallholder production system, as well as the lack of resources and technologies for genotyping [23].

Three main factors are decisive to design a reliable GWAS: i) accurate phenotype and genotype data, ii) sufficient sample selection and sample size, and iii) application of adequate statistical methods [24]. With regard to further challenges such as population stratification, environmental influences and the complexity of quantitative traits, statistical methods are gradually enhanced [25, 26]. The broad availability of open-source software packages implementing innovative methods such as PLINK [27] and GCTA [28] for genome-wide complex trait analyses, in combination with continuously declining genotyping costs, open the potential for pilot GWAS in unstudied and undeveloped breeds for novel traits. Nevertheless, the recording of accurate phenotypes remains one big challenge in the African livestock-breeding context. The Food and Agriculture Organization of the United Nations (FAO) guidelines highlight morphometric traits as a good starting point to initially characterize unstudied breeds phenotypically and genetically [29]. Actually, morphometric traits comprise linear measurements of animal body sizes. Morphometric traits are routinely measurable even at early ages, and are proper early indicators for animal growth, health, welfare, and longevity [30–32]. Moreover, because of the accurate measurements (mostly in cm), morphometric traits provide an objective and better assessment of body traits than subjectively conformation traits scoring [33–35].

Several GWAS associated single nucleotide polymorphism (SNP) and genes to cattle morphometric or conformation traits. Major and commonly investigated cattle body traits for GWAS were hip height (stature), height at withers, body length, hip (rump) width, chest width, and scrotal circumference [36–38]. The heritabilities generally reported for these traits were moderate to large [39–41]. Moreover, despite the fact that the genomic architecture of cattle body traits is highly polygenic, many similarities with other livestock species, human and mammals in general, were observed [42, 43]. The identified regions were mainly involved in biological functions such as regulation of fetal growth, skeletal development, regulation of cell cycle or cell division, homeostasis, and lipid metabolism [40–44]. More interestingly, different studies identified significant overlaps in genomic architecture, and in genomic relationships between morphometric or conformation traits with cattle performance traits including body weight, carcass trait, feed intake, reproduction and health [42–46]. Indeed, phenotypic correlations between morphometric traits and animal performances such as body weight and milk offtake were very similar in various African breeds [47–49]. Furthermore, models to predict body weight from heart girth, body length or height at withers have been established [47, 48], suggesting morphometric traits as major predictors of animal performances in African livestock herds, where recording systems are poorly developed. Addressing principles of selection, Kabi et al. [50] indicated that morphometric population diversity is a result of selection for adaptive and sociocultural interests in African smallholder livestock production context. Hence, investigating genomic regions associated with morphometric traits is worthwhile in African breeds, as it allows a better understanding of animal diversity and adaptation features. In addition, its opens prospects for the effective use of morphometric traits in basic phenotype recording for any potential community-based breeding program in African smallholder livestock systems.

As discussed by many scientists [24, 51], GWAS are exploratory in their nature and further investigations based on SNP marker effects are required to get more insights into the genetic and biological basis of a trait. Hou and Zhao [52] reviewed tools and genomic features such as differential gene expressions, protein deleteriousness predictions and DNase I hypersensitive sites that could be used in understanding biological causal mechanisms and the functional relevance of identified significant SNP. In the context of limited resources, approaches that rely on documented information and public databases, such as candidate gene functional annotation and enrichment analyses of gene ontology (GO), even though non-analytical, offer new prospects for a deeper interpretation of results from GWAS [53].

Cattle in Benin are commonly kept in small herds under extensive production conditions. The indigenous cattle breeds of Benin consist of two taurine (Somba and Lagune) and two hybrid (taurine x indicine, i.e., Borgou and Pabli) breeds. These breeds were described for their adaptive potential to disease and harsh environmental conditions as well as for their importance in the livelihoods of poor farmers [5, 14, 54, 55]. However, due to their low productivity, they are increasingly threatened by indiscriminate crossbreeding with zebu animals [5, 56]. In addition, routine performance recordings and structured breeding programs have not been developed yet. The existing threats were confirmed in a genetic diversity approach considering indigenous cattle breeds from Benin, focusing on the effects of transboundary transhumance [57]. The present study builds on the dataset established in Scheper et al. [57], and combines 50 k SNP data with a basic phenotypic characterization according to FAO guidelines [29]. Given the threats surrounding the indigenous cattle breeds in Benin and in Sub-Saharan Africa in general, genetic evaluations based on marker data are an important first step to develop sustainable conservation and breeding strategies.

The aim of the present study was to estimate genetic parameters and to perform genome-wide associations for morphometric traits in four indigenous cattle breeds from different agro-ecological zones (AEZ) of Benin using medium density SNP chip data. In addition, functional annotation and gene enrichment analyses were applied to identify genes and functional loci potentially associated with morphometric traits. Finally, the indigenous Benin breeds from smallholder farms were contrasted genomically with other African livestock and exotic breeds or crossbreeds raised under improved management conditions in research stations [58, 59].

Results

Heritabilites, phenotypic and genetic correlations

SNP-based heritability estimates for the morphometric traits ranged between 0.46 ± 0.14 (HG) and 0.74 ± 0.13 (HW, EL, Table 1). Heart girth showed the largest genetic correlations (r_g) with all other morphometric traits (0.38–0.80). Overall, genetic correlations among all

Table 1 Estimated phenotypic and genetic correlations among morphometric traits and their heritability. Heritabilities (in bold) are on the diagonal, above the diagonal are the genetic correlations and below the diagonal are the phenotypic correlations

	HAW	**SH**	**HG**	**HW**	**BL**	**EL**
HAW	**0.72 ± 0.08**	0.85 ± 0.02	0.80 ± 0.28	0.46 ± 0.05	0.25 ± 0.08	0.42 ± 0.11
SH	0.89 ± 0.01	**0.70 ± 0.08**	na	0.46 ± 0.05	0.19 ± 0.09	0.42 ± 0.13
HG	0.62 ± 0.03	0.59 ± 0.03	**0.42 ± 0.14**	0.72 ± 0.15	0.38 ± 0.15	0.58 ± 0.25
HW	0.50 ± 0.04	0.49 ± 0.04	0.58 ± 0.03	**0.74 ± 0.13**	0.14 ± 0.10	0.35 ± 0.17
BL	0.37 ± 0.04	0.32 ± 0.04	0.45 ± 0.04	0.25 ± 0.05	0**.73 ± 0.10**	0.25 ± 0.10
EL	0.35 ± 0.04	0.33 ± 0.04	0.29 ± 0.04	0.25 ± 0.05	0.33 ± 0.04	**0.74 ± 0.10**

HAW height at withers, *SH* sacrum height, *HG* heart girth, *HW* hip width, *BL* body length and *EL* ear length morphometric traits ranged from 0.14 (HW with BL) to 0.85 (HAW with SH). Most of the estimated genetic correlations had small standard errors below 0.11. The largest standard error (SE = 0.28) was estimated for the genetic correlation between HG and HAW. In general, HG had larger SE for genetic correlations and for the heritability in comparison to all other traits. The phenotypic correlations (r_p) ranged between 0.25 ± 0.05 (HW with BL) and 0.89 ± 0.01 (HAW with SH). Heart girth and hip width were phenotypically and genetically highly correlated ($r_p = 0.62$, $r_g = 0.72$). In contrast, phenotypic and genetic correlations between EL and BL ($r_p = 0.33$, $r_g = 0.25$) were considerably lower. In addition, BL showed the smallest phenotypic and genetic correlations with other traits, especially with HW ($r_g = 0.14–0.38$, $r_p = 0.25–0.45$).

Multi-breed GWAS for morphometric traits and functional annotation of candidate genes

Discriminant analysis of principal components (DAPC) identified four linear discriminant functions (LDF) validly representing the genetic structure in the sampled population (Additional file 1, Figure S1). The inclusion of the LDF in the PLINK GWAS resulted in sufficient correction of population stratification with desired lambda values ($\lambda = 0.99–1.09$). Slightly lower lambda values ($\lambda = 0.98–1.00$) were obtained from GWAS using GCTA (Fig. 1). The GWAS via PLINK detected a total of 28 SNP for all six morphometric traits, and the majority of these SNP were also detected via GCTA (Fig. 1). The significant SNP from PLINK were positioned within or near 15 different genes (Table 2).

The significant SNP, potential candidate genes and their functional annotation are presented in the ongoing sub-chapters for the morphometric traits, considering two traits per sub-chapter.

Height at withers and sacrum height

Height at withers was significantly associated with the SNP *rs109126926* on BTA1 (*p* = 1.12e-06). In addition, five chromosome-wide suggestively associated SNP were detected on chromosomes 3, 17, 19 and 21 (*p* = 6.44e-06 - 5.04e-05); see Table 2 for the exact positions). The SNP *rs110369628* and *rs109889052* on BTA19 were positioned in relative proximity to each other (7.41 Mb), within the *PIK3R6* and *SSH2* genes, respectively. The *VEPH1* gene harbored the significantly associated SNP for HAW on BTA1, while another SNP, *rs4163436*, was positioned in *CCDC117* on BTA17. No genes were annotated for the two remaining significantly associated SNP.

Only two SNP were suggestively associated with SH (*p* = 2.35e-05 - 3.86e-05) and each was located in close distance to a gene. The SNP *rs111001850* was positionally linked to the *LYPD8* gene on BTA7, and the SNP *rs110441360* was located near the *PIK3R1* gene on BTA20.

Heart girth and hip width

No SNP surpassed the genome-wide significance threshold for associations with HG and HW. However, four SNP were suggestively associated with HG (*p* = 1.17e-05 - 4.10e-05). Two of them were positioned on BTA2 and the two others were located on BTA19 and BTA22. The SNP *rs41579167* on BTA2 was positioned near the two genes *PTAFR* and *EYA3*. The gene *PBRM1* harbored the SNP *rs41637645* on BTA22.

Hip width was associated with three suggestive SNP (*p* = 2.08e-05 - 2.99e-05). Two of the three SNP were positioned relatively near to each other (4 Mb) on BTA16. The third SNP was detected on BTA8. Only the *ABL2* gene was mapped as a potential candidate gene for HW, harboring the SNP *rs42843320* on BTA16.

Body length and ear length

Body length presented the highest number of associated SNP with two significant and 7 suggestive SNP. BTA2 harbored one significant SNP (*rs110694334, p* = 8.19e-07*)* along with two others suggestive SNP (*p* = 9.33e-06 - 1.38e-05). Similarly, BTA21 harbored the second significant SNP (*rs41607390, p* = 2.03e-07) and one suggestive SNP (*p* = 3.74e-05), but none of them was positionally linked to a gene. The other associated SNP variants were identified on chromosomes 3, 10, 17 and 27 (*p* = 1.91e-05 - 4.93e-05). On BTA2, the SNP *rs110694334* was positioned within the *CNTNAP5* gene, in relative proximity (2.32 Mb) to the SNP *rs109186122* positioned in the *GYPC* gene. Two additional genes, *SLC16A4* and *MEGF11*, were mapped as potential candidates for BL, harboring the SNPs *rs135705191* and *rs43616983* on BTA3 and BTA10, respectively.

Four SNP positioned on different chromosomes (7, 10, 12, 20) were suggestively associated with EL (*p* = 1.08e-05 - 2.54e-05). The SNP *rs110608572* SNP was positioned on BTA10 within the *MEGF11* gene, which was also identified as a potential candidate gene for BL. The SNP *rs109985119* was located in the *ADAMTS12* gene on BTA20.

Discussion

Heritabilities, phenotypic and genetic correlations

The heritability estimates for morphometric traits obtained in the present study were larger than those commonly reported for comparable linear body traits [39–41, 60–63]. However, heritability estimates are usually higher for morphometric traits (measurements) [39–41] than for conformation traits (scores) [60, 61, 63]. Hence, quite large heritability estimates (up to 0.7) have been similarly reported in few studies based on morphometric traits [64–67]. For instance, the heritability estimate of 0.42 for HG in our study is comparable to the value of 0.43 reported for adult Brahman cattle [66]. Moreover, the heritabilities of 0.72 for HAW and of 0.70 for SH are

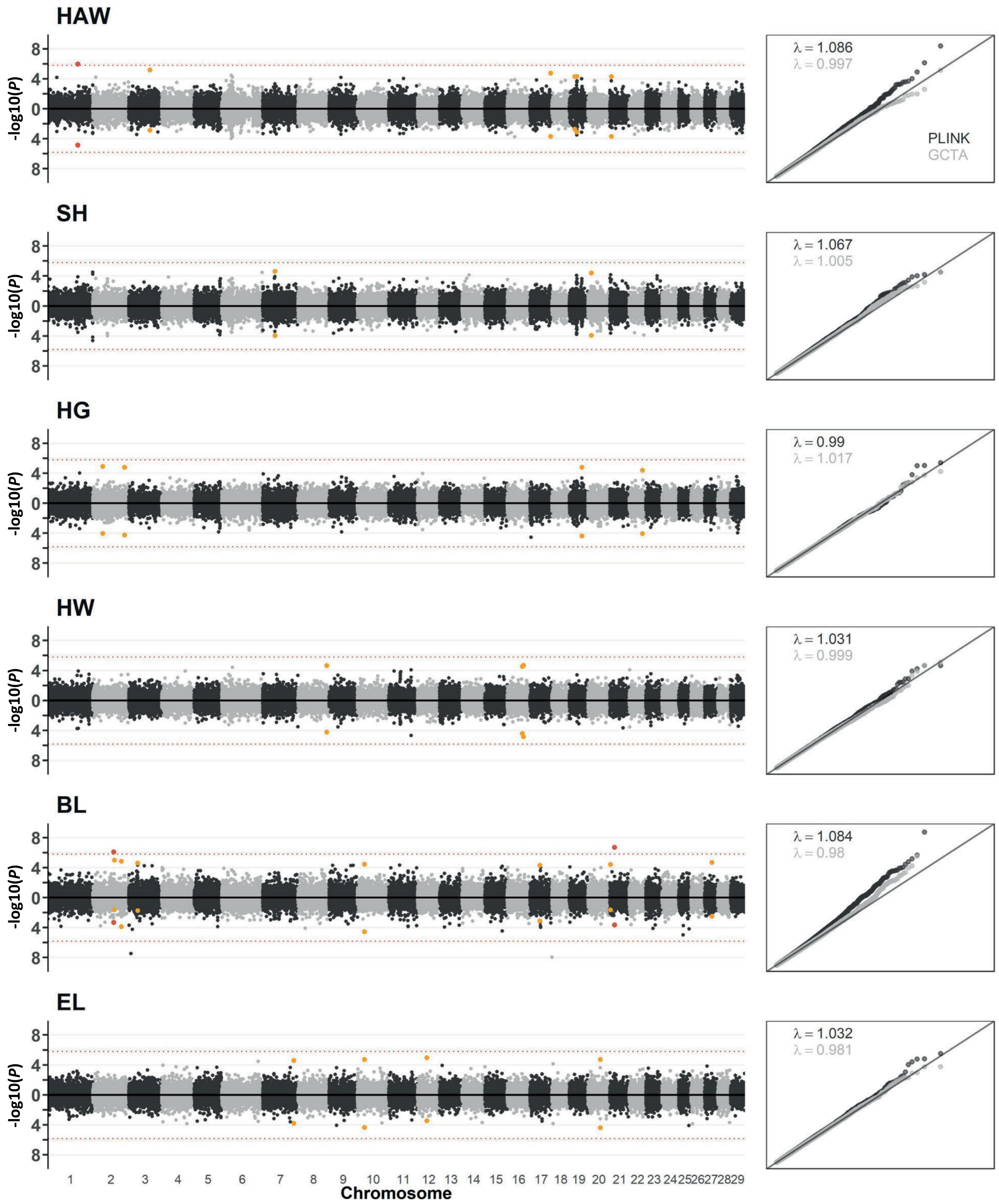

Fig. 1 Manhattan Plots and QQ-plots displaying GWAS results from PLINK (above the x-axis) and GCTA (below the x-axis) for six morphometric traits in four indigenous cattle breeds from Benin. The genome-wide significant SNP and chromosome-wide significant SNP are displayed in red and yellow, respectively. HAW = height at withers, SH = sacrum height, HG = heart girth, HW = hip width, BL = body length and EL = ear length

Table 2 Genome-wide and chromosome-wide significant SNP and potential candidate genes associated with six morphometric traits from four indigenous breeds in Benin

SNP_rs	SNP	CHR	BP_ARS1.2	Alleles		MAF	BETA		*P*-value		SNP in Gene	Gene Name
				A1	A2		PLINK	GCTA	PLINK	GCTA		
Height at withers												
rs109126926[a]	ARS-BFGL-NGS-57889	1	110,160,486	A	G	0.26	2.24	2.16	1.12e-06	1.44e-05	Yes	*VEPH1*
rs43347748	ARS-BFGL-NGS-31952	3	81,139,242	C	T	0.10	2.60	1.96	6.44e-06	1.40e-03	–	–
rs41634361	Hapmap50686-BTA-41836	17	68,037,062	G	A	0.10	2.58	2.40	1.71e-05	1.99e-04	Yes	*CCDC117*
rs110369628	ARS-BFGL-NGS-91812	19	20,892,297	T	C	0.02	5.58	4.55	4.82e-05	2.00e-03	Yes	*SSH2*
rs109889052	ARS-BFGL-NGS-81151	19	28,301,046	A	G	0.13	2.35	2.08	4.57e-05	7.94e-04	Yes	*PIK3R6*
rs109872376	ARS-BFGL-NGS-46597	21	7,674,101	C	G	0.44	1.62	1.54	5.04e-05	2.09e-04	–	–
Sacrum height												
rs111001850	ARS-BFGL-NGS-7310	7	42,545,291	A	G	0.45	1.53	1.50	2.35e-05	1.12e-04	No	*LYPD8*
rs110441360	ARS-BFGL-NGS-110086	20	11,381,378	A	G	0.48	−1.51	−1.51	3.86e-05	1.19e-04	No	*PIK3R1*
Heart girth												
rs110404606	ARS-BFGL-NGS-77689	2	32,163,209	C	T	0.38	2.83	2.55	1.17e-05	9.65e-05	–	–
rs41579167	BTA-49621-no-rs	2	125,244,296	G	A	0.44	−2.93	−2.75	1.55e-05	5.59e-05	No	*PTAFR* *EYA3*
rs41624005	Hapmap48676-BTA-18047	19	46,729,603	A	G	0.50	2.72	2.64	1.60e-05	4.29e-05	–	–
rs41637645	Hapmap39844-BTA-54797	22	48,208,654	C	A	0.16	−3.48	−3.36	4.10e-05	8.96e-05	Yes	*PBRM1*
Hip width												
rs109866742	ARS-BFGL-NGS-119529	8	103,907,956	T	C	0.02	−3.12	−3.06	2.11e-05	5.89e-05	–	–
rs41569598	BTA-39611-no-rs	16	56,426,595	C	T	0.43	−0.97	−0.97	2.99e-05	3.88e-05	–	–
rs42843320	BTB-01732320	16	60,525,984	C	T	0.22	−1.12	−1.15	2.08e-05	1.47e-05	Yes	*ABL2*
Body length												
rs110694334[a]	ARS-BFGL-NGS-109828	2	76,610,609	C	T	0.03	−17.96	−11.94	8.19e-07	4.90e-04	yes	*CNTNAP5*
rs109186122	ARS-BFGL-NGS-118432	2	78,925,610	C	T	0.25	−7.18	−3.43	9.33e-06	2.48e-02	yes	*GYPC*
rs42301516	BTB-01145402	2	113,155,761	G	A	0.43	−5.45	−4.31	1.38e-05	1.37e-04	–	–
rs135705191	BovineHD0300010335	3	33,048,892	G	A	0.20	−6.85	−3.72	2.49e-05	1.90e-02	yes	*SLC16A4*
rs43616983	BTB-00409355	10	12,969,418	G	A	0.20	6.70	6.20	3.41e-05	2.94e-05	yes	*MEGF11*
rs42436268	BTB-01308172	17	33,487,976	G	T	0.32	5.45	4.06	4.93e-05	8.18e-04	–	–
rs41608167	BTA-96370-no-rs	21	3,408,269	T	C	0.02	−18.36	−9.53	3.74e-05	2.29e-02	–	–
rs41607390[a]	Hapmap33092-BTA-51753	21	18,775,375	T	C	0.11	−10.39	−7.07	2.03e-07	2.20e-04	–	–
rs41646754	Hapmap44720-BTA-62525	27	24,546,315	G	C	0.27	−6.28	−4.04	1.91e-05	3.27e-03	–	–
Ear length												
rs109212458	ARS-BFGL-NGS-85383	7	96,266,192	T	C	0.33	0.42	0.38	2.54e-05	1.63e-04	–	–
rs110608572	ARS-BFGL-NGS-103122	10	12,919,428	A	G	0.03	1.10	1.07	1.86e-05	4.40e-05	Yes	MEGF11
rs41567897	BTA-100327-no-rs	12	41,375,572	C	G	0.17	−0.59	−0.49	1.08e-05	3.56e-04	–	–
rs109985119	ARS-BFGL-NGS-44763	20	40,060,386	T	C	0.14	0.56	0.54	1.90e-05	4.23e-05	Yes	ADAMTS12

[a] Genome-wide significant SNP; the remaining SNP are the chromosome-wide significant SNP

in agreement with the estimates for stature in Brahman (0.73) [65] and in Red & White cattle (0.74) [64]. These observations suggest that real and objective measurements provide a better basis for heritability estimations than subjective conformation scores.

The estimates of genomic correlations for the different trait combinations ranged from 0.14 to 0.84, with standard errors between 0.02 to 0.25. Roveglia et al. [63] reported a broad range (0.08 to 0.98) for genetic correlations among conformation traits reflecting the morphometric characteristics from the present study. The genetic correlation between HW and height traits (0.46) in the present study was smaller than the estimate of 0.75 [63]. Nevertheless, generally lower genetic correlations (0.09 to 0.35) between these trait combinations have been previously described [64, 68]. The genetic correlation of 0.80 between HG and HAW from the present study is larger than the values that were previously reported (0.30–0.65) [40, 64, 69, 70]. The large genetic correlations between HG with the other morphometric traits are in agreement with results from a previous study [64].

The estimates of phenotypic correlations between height traits (HAW and SH) and HG (0.59 and 0.62) were slightly larger than those (0.20 to 0.50) reported in previous studies [40, 70]. However, regarding the comparisons made, it is imperative to state that our study is the only one using genomic instead of pedigree relationships. To our knowledge, this is the first study, which estimated phenotypic and genetic correlations among morphometric or conformation traits on the basis of SNP marker data. Moreover, the cited genetic parameters estimates used for the comparisons are from Holstein, Brahman, Brown Swiss and Red & White populations kept in Europe or Asia [40, 63–66]. The few genetic evaluations in the African context were made for growth traits (birth, weaning and yearling weight) in exotic breeds kept under controlled management conditions in research stations [58, 59]. In African smallholder farms, genetic parameters only have been estimated for milk production, considering exotic breeds and crossbreeds (exotic × indigenous) [71].

As a major difference to previous studies, our approach focused on multi-breed genetic parameter estimations. Meyer et al. [72] observed larger heritabilities and genetic variances in multi-breed populations, compared with estimates in purebred populations. These findings are in agreement with the moderate to high values for genetic parameters obtained in our study. However, high genetic variability also was identified in single breeds, but potentially be biased due to correlations between environments and genotypes, and due to extremely close genetic relationships [73]. Thus, accounting for population structure particularities remains a great challenge in all studies estimating genetic parameters, even within a single breed [73]. Our study attempted to avoid relatedness in the dataset by sampling animals from different herds and AEZ. Nevertheless, breed admixture was oberved between and within the indigenous breeds in Benin, due to continuous indicine introgression and uncontrolled crossbreeding [57]. Thus, breed specific modelling approaches might be affected from within-breed diversity. Overall, the heritabilities, phenotypic and genetic correlations from the present multi-breed study reflect the range for single-breed estimates, indicating that most of the genetic variations in body traits is based on universal common rather than on breed specific genetic variants [74]. Indeed, multi-breed population references enhance the potential in detecting common and conservative loci [72, 75]. In this regard, Wientjes et al. [76] focused on model comparison, and observed most accurate genetic parameters from models capturing large proportions of the genetic variance in a population. Therefore, in the context of uncontrolled crossbreeding as prevalent in indigenous cattle breeds in Africa, a multi-breed approach might be useful.

Multi-breed GWAS for conformation traits and functional annotation of candidate genes

Height at withers and sacrum height

For the SNP *rs109126926* SNP significantly associated with HAW and the corresponding gene *VEPH1*, no direct association between the variant or the identified gene with cattle height were previously reported. However, *rs109126926* was significantly associated with recoverability from mastitis in Holstein cows [77]. *VEPH1* influenced other cattle conformation traits such as udder cleft in Holstein and rump fat thickness in Nellore cattle, reflecting its role in lipid metabolism [78, 79]. Furthermore, recent studies observed an association of *VEPH1* with residual feed intake and antibody response to parasites in cattle [80, 81].

Among the five suggestive SNP associated with HAW, *rs109889052* and *rs110369628* were located on BTA19. These two SNP have not been directly associated with cattle height, but *rs109889052* contributed to feed efficiency in cattle [82]. Moreover, *rs109889052* is located in the *PIK3R6* gene, affecting body size in sheep [31]. Likewise, *rs110369628* positioned in the *SSH2* gene, was linked to carcass traits in sheep [83]. According to the database for annotation, visualization and integrated discovery (DAVID), *SSH2* is involved in the regulation of the actin cytoskeleton pathway and in several other molecular and biological functions such as the regulation of actin polymerization or depolymerization and DNA binding. In previous studies, *SSH2* was associated with somatic cell score and semen traits in cattle [84, 85]. Bouwman et al. [42] identified several SNP on BTA19 associated with cattle stature, including *rs132693733* (19:21339030), *rs109018020* (19:23816722) and *rs137732346* (19:25980624). These markers are positioned in a distance between 0.45 Mb and 6.96 Mb from the SNP identified in our study, i.e., *rs109889052* (19:20892297) and *rs110369628* (19:28301046). Another variant *rs42741630 (*19:25439551) in close proximity (2.86 Mb) to *rs110369628* (19:28301046), was detected in a recent GWAS for stature [86]. These findings suggest that this chromosomal segment on region BTA19 (at 20 Mb to 30 Mb) might represent a hotspot genomic region for height. We found no reference for the three other variants associated with HAW in the literature. Nevertheless, the SNP *rs41634361* on BTA17 is positioned within the *CCDC117* gene, which is involved in feed intake and heat stress regulation in cattle [87, 88].

The two SNP suggestively associated with SH were not associated with cattle height in previous studies. However, the SNP *rs110441360* on BTA20 is positioned in the *PIK3R1* gene, which influenced beef fat content [89]. More interestingly, different studies described *PIK3R1* for its implication in the human short stature syndrome [90, 91], confirming the functional conservation of genes linked to stature or body size in cattle and humans [42–44]. Moreover, these findings are consistent with the functional annotation of *PIK3R1* in protein stabilization, insulin resistance, and growth hormone receptor signaling pathways. The potential effect of growth hormone receptor genes on animal conformation traits has been previously reported [92]. The second variant (*rs111001850)* associated with SH is located in the *LYPD8* gene. This gene is involved in biological processes of defense responses to gram-negative bacteria, and has been related to adaptive responses to environmental stimuli such as stress, infection and inflammation in cattle [93].

The association of two PIK regulatory subunit genes with height traits in this study is remarkable. Another *PI3K regulatory subunit* gene, i.e.*PIK3R5*, was identified in a region highly associated with body size in sheep [31]. Moreover, *PIK3R6* and *PIK3R1* are declared as potential candidates for feed intake and feed efficiency in cattle [82, 94]. Both genes are members of a metabolism pathway, specifically involved in the synthesis of PIPs at the plasma membrane. In addition, we found that *PIK3R6* and *PIK3R1* are involved in several animal immune system pathways or related biological processes, such as the B cell receptor signaling pathway, the AMPK signaling pathway and regulation of T cell differentiation. Moreover, they are members of different signal transduction pathways (G beta: gamma signaling through PI3Kgamma, signaling by SCF-KIT), which are also linked to inflammatory diseases [95, 96]. The involvement of *PIK3R6* and *PIK3R1* in signal transduction and immunity pathways reflect the associations with resistance to *Mycobacterium avium ssp. paratuberculosis* (MAP) infections in cattle [27–29].

Indigenous cattle breeds in Benin, especially the taurine breeds, are known to be resistant against diseases [14, 54, 55]. In comparison to other breeds, they are small sized, and kept in harsh environments with limited feed resources and a high disease infection risk. In this context, HAW is described as an indicator of animal adaptive attributes [29]. Hence, our findings for HAW and SH confirm that *PIK3R6* and *PIK3R1* may play an important role in the interaction of adaptation to diseases and body size variability in African cattle, and potentially in cattle populations worldwide.

Heart girth and hip width

The four SNP suggestively associated with HG were not detected in any previous GWAS for HG. However, two of them are positionally linked to three genes influencing dairy cattle traits. First, the SNP *rs41579167* SNP is located in direct proximity to the genes *PTAFR* and *EYA3.* These two genes are involved in inflammatory responses and both are associated with immune response in cattle [97, 98]. In addition, *PTAFR* is associated with intramuscular fat deposition in Nellore cattle [99] and was identified in selective regions for production performance in different cattle breeds [100, 101]. Furthermore, in mice, *PTAFR* affected body weight by controlling feed intake and obesity [102]. According to DAVID, the *EYA3* gene is involved in cell differentiation processes and in a pathway related to DNA repair mechanisms. This gene was also identified as a potential candidate gene in a GWAS for milk production in dairy sheep [103]. The SNP *rs41579167* SNP (2:125244296) is located at 0.15 Mb distance to another variant *rs2083797338* (2:125093797), which has been identified in a meta-GWAS for cattle stature [42].

Second, the SNP *rs41637645* is located within *PBRM1,* a gene which was associated with heat stress regulation in tropical breeds [104]. *PBRM1* is also involved in the negative regulation of cell proliferation and in the RMTs methylate histone arginine pathway, while this pathway is involved in different diseases in mammals [105].

We found no direct link between the four suggestively associated SNP for HW with HW in previous studies. Nonetheless, *ABL2* is a potential candidate gene for HW, harboring *rs42843320*. *ABL2* influenced feed intake in cattle and backfat thickness in pigs [106, 107]. In addition, *ABL2* is involved in innate immune response processes through cell proliferation, migration and differentiation (according to its functional annotation in the DAVID database).

Body length and ear length

Body length was significantly associated with *rs41646754* on BTA27, and with *rs110694334*, which is located within *CNTNAP5* on BTA2. Neither the two variants, nor the identified genes, have been previously associated with BL. Nevertheless, *CNTNAP5* was detected in a chromosomal segment significantly associated with hip cross height in Brahman cattle [108]. Furthermore, *CNTNAP5* is a potential candidate gene for conformation traits in Sudanese goats and growth traits and pigs [109–111]. In addition, *CNTNAP5* was identified in a selective region for adaptation in cattle as well as in sheep [112, 113]. According to DAVID, *CNTNAP5* is an integral component of membranes and is related to the epidermal growth factor-like protein domain.

Among the seven suggestive SNP associated with BL, *rs43616983* on BTA10 was associated with direct perinatal mortality in Holstein–Friesian dairy cattle [16]. Moreover, *rs43616983* is positioned within the *MEGF11* gene, which regulated daily gain and immune response to mastitis in cattle [85, 114]. Another study observed different expressions of *MEGF11* in the *musculus longissimus dorsi* of two different cattle breeds in response to low energy diets [85]. This finding is consistent with results from GWAS in pigs suggesting *MEGF11* as a potential candidate gene for feed efficiency [115]. Furthermore, *MEGF11* was significantly associated with height in Buffalo [116]. Two other SNP suggestively associated with BL, *rs109186122* and *rs43616983*, are located in the genes *GYPC* and *SLC16A4*, respectively. According to its annotation in DAVID, *GYPC* is involved in oligosaccharide binding and is reported to be associated with intramuscular fat deposition and reproduction performances in cattle [117–119]. The *SLC16A4* gene had effects on postweaning weight gain, feed efficiency and resistance to the bovine viral diarrhea virus in cattle [120–122]. These observations are in line with the involvement of *SLC16A4* in transmembrane transport functions and glucose import processes, as well as with the impact of the SLC16 gene family on health regulation [123]. The *SLC16A4* gene is located in a genomic segment being under divergent selection in South African cattle breeds [13]. In addition, solute carrier family genes were associated with cattle body weight or conformation traits [124, 125].

None of the four SNP variants suggestively associated with EL are linked to EL in the literature, because of the trait relevance only for tropical production systems. Two potential candidate genes for EL are related to different traits in cattle and other mammal species. Firstly, *MEGF11*, harboring the SNP *rs110608572* on *BTA10,* overlap with our findings for BL. Secondly, *ADAMTS12,* harboring the SNP *rs109985119* on BTA20, was associated with body weight and supernumerary teat in cattle [126–128]. *ADAMTS12* was involved in inflammatory responses and in the regulation of the hepatocyte growth factor (HGF) receptor signaling pathway. Indeed, HGFs are known to play an important role in the stimulation of epithelial cell proliferation, motility, morphogenesis and angiogenesis [129]. The *ADAMTS12* gene was identified in pigs in a selection signature associated with genetic adaptation to high altitude [130]. These observations confirm a potential effect of *ADAMTS12* on EL variability, considered as an adaptive trait for heat tolerance in the indigenous breeds in Benin [29, 131].

Genomic regions associated with morphometric and adaptation traits

The comparison with the literature gives convincing evidence for the validity of our results based on gene functions and associations as detected in other traits and species. The rather small number of detected SNP for the moderately to highly heritable polygenic traits is in line with our small sample size and its diversity (multi-breed), and is most likely due to a lack of power to detect variants with smaller effects. However, with regard to the functional annotations of the identified genomic regions, our study confirms the potential of multi-breed GWAS in detecting fewer variants, but more precise functional loci or causative mutation across breeds [75, 132]. In this context, the SNP associated with morphometric traits in the present study may represent novel common variants for linear body traits in African breeds, suggesting further investigations. Furthermore, the validity of the multi-breed approach may constitute to collaborative research towards better characterizations and genetic evaluations, including the animal genetic resources in Africa [2, 3, 71, 133].

Our findings reveal a close connection between genomic regions associated with morphometric traits and adaptive traits. For instance, two of the identified variants detected for morphometric traits in this study are directly linked to immune response, while several potential candidate genes have functional annotations for, e.g., signal transduction, metabolism, and immune response adaptation [134]. The comparison with previous studies confirms associations of the identified genes with selection signatures as well as immunity or resistance to diseases, feed efficiency and adaptation to harsh environments (heat stress, high altitude), addressing the main components of adaptation [4]. On the one hand, our observations are consistent with the breeding history of the indigenous breeds in Benin, which is characterized by natural and non-directional selection based on individual farmer preferences. In many African breeds, adaptive traits (disease resistance, feeding ease) and reproductive performances reflect the major breeding preferences of farmers, and they select animals according to their morphometric or conformation appearance rather than on actual recorded performance [50, 135, 136]. Hence, this might be an explanation for the observed association between morphometric and adaptive traits in our study [50, 137].

On the other hand, genomic regions associated with adaptive traits (immune response or feed efficiency) have been similarly detected in GWAS for linear body traits in more developed breeds such as Holstein and Angus [36, 62, 92, 138]. These overlaps as well as the effect of selection response for disease resistance or feed efficiency on conformation or carcass traits, and vice-versa, have been extensively discussed [138, 139]. In our view, two hypotheses emerge from such overlap. First, the identified loci or genomic regions may simultaneously control morphometric traits and adaptive traits, indicating the pleiotropic role of many loci associated with body conformation traits such as height or body size [42]. Secondly, a high genetic correlation between morphometric and adaptive traits is due to the long history of natural selection in the studied breeds on both trait categories.

Conclusion

Heritabilities for as well as phenotypic and genetic correlations among morphometric traits based on dense SNP marker data and a multi-breed approach were moderate to moderately high. Twenty-five SNP and fifteen genes potentially associated with the morphometric traits were detected. Comparisons with previous studies and the functional annotation of the genes revealed a clear association of loci identified in this study with conformation, growth and carcass traits in cattle or in other species. Moreover, the majority of the detected genes are associated with immune response and feed efficiency, or involved in related biological processes, suggesting a strong correlation between morphological and adaptive traits. This is in line with the evolutionary development and breeding history in these indigenous breeds mainly shaped by natural selection. Our findings suggest that accurate phenotyping (measurements) for morphometric traits combined with SNP marker data can be used for genetic evaluations, considering mixed-breed cattle populations.

Methods

Phenotypes and animal resources

Following the FAO guidelines [29], we recorded six morphometric traits (Table 3) on 449 animals from the four main indigenous cattle breeds in Benin (Borgou 181, Pabli 58, Lagune 150, Somba 60). The morphometric traits were chosen for their importance in cattle characterization and their association with production or adaptive traits. Sacrum height, heart girth, hip width and body length are commonly used to evaluate cattle body size and growth, and they are highly correlated with body weight or milk yield in different cattle breeds [40, 44, 47, 48, 66, 140]. In addition, ear length and height at withers are related to adaptive traits, whereas hip width is associated with animal longevity [29, 30].

The animals were selected from a larger dataset of 462 animals as described in a previous study [57]. Thirteen animals from the larger dataset were excluded from the current study due to inconclusive genetic adherence and impact from crossbreeding [57]. The animals were sampled in small cattle herds kept under extensive management, according to their distribution across AEZ in Benin (see Additional file 2, Table S1 for animal characteristics, location and geographic coordinates of the herds). Somba and Pabli cattle were sampled in the AEZs Ouest Atacora (OA) and Cotonnière Nord (CNB). Borgou cattle were sampled in three different AEZs Cotonnière Centre (CCB), Vivrière Sud Borgou (VSB) and Cotonnière Nord (CNB), and the Lagune cattle in three other AEZs Pêcheries (P), Dépression (D) and Terre de Barre (TB). One-way analysis of variance (ANOVA) tests for the six morphometric traits revealed significant differences *($p \leq 0.001$)* across breeds with higher values for hybrid breeds (Borgou and Pabli). For instance for height at withers, Borgou and Pabli cattle measured 116.5 ± 5.67 cm and 111.4 ± 7.58, but Somba and Lagune are smaller with 96.37 ± 4.97 cm and 92.59 ± 7.18, respectively. The full description of the six morphometric traits for the four breeds are presented in Additional file 3, Figure S2.

Genotypes, quality control and imputation

The 449 selected animals with phenotypes were genotyped using the Illumina BovineSNP50 BeadChip, and 51,278 SNP were available before quality control. Sample collection, DNA extraction and genotyping procedures are described in detail in Scheper et al. [57].

Genotyping quality control was performed using the PLINK software [26] to retain SNP with a minor allele frequency larger than 5% and a genotyping call rate of 90%, and which are in Hardy-Weinberg equilibrium ($p \geq 10^{-06}$). For individual animals, a genotype call rate larger than 95% was required. After quality control, 14,518

Table 3 Description of the six morphometric traits recorded on four indigenous cattle breeds from Benin

Morphometric traits	Description	Measuring device
Height at withers	Vertical distance from the bottom of the front foot to the highest point of the shoulder between the withers	Measuring stick
Sacrum height	Distance from the top of the bone at the base of the tail to the ground	Measuring stick
Heart girth	Circumference of the body immediately behind the shoulder blades in a vertical plane, perpendicular to the long axis of the body	Measuring tape
Hip width	Distance between the rearmost posterior points of pin bones	Wooden caliper
Body length	Horizontal distance from the point of the shoulder to the pin bone	Measuring tape
Ear length	Length on the back side of the ear from its root on the poll to the tip	Measuring tape

SNP and one animal were discarded, implying a genotype dataset with 36,760 SNP variants from 448 cattle.

Sporadic missing variants were imputed with BEAGLE [141] after remapping the SNP positions to the current reference assembly ARS1.2 and removing all markers with unknown position on ARS1.2. Genotype imputation generated a dataset of 36,720 SNP for the ongoing genomic analyses.

Adjustment for fixed and environmental effects and genetic structure

One-way ANOVA in R was firstly applied on each morphometric trait to test the explanatory variables for significance. Given that breeds are nested within AEZ, a new variable (AEZ_Breed), which combines the two variables AEZ and breed, was created. The factors age, sex, and AEZ_Breed were simultaneously included in a multi-factor linear model to test their effects on the respective morphometric trait via the Type III sums of squares from ANOVA, using the Car package in R [142]. AEZ_Breed showed significant effects on the six morphometric traits *($p \le 0.001$)*, while sex had significant effect only on HG, HW and BL, and age on HG, HW and HAW (Additional file 4, Table S2). In consequence, AEZ_Breed, sex and age were considered as fixed effects in the GWAS models for all morphometric traits.

The genetic structure in the dataset was evaluated applying a DAPC, using the R package ADEGENET [143, 144]. LDF were used as covariates in addition to AEZ_Breed, sex and age to correct for population stratification in GWAS. DAPC was chosen instead of classical principal component analysis (PCA) as it better characterizes the genetic structure of the population [145, 146] (see Additional file 4, Table S3, for significant SNP when the first four PCs were included as covariates in the GWAS model). The genotype dataset used for DAPC consisted of 25,065 SNP after pruning of the imputed dataset based on linkage disequilibrium (LD) between markers. The *"--indep-pairwise"* command in PLINK and defining 0.2 for the r^2 threshold, was considered in this regard [27].

Estimation of heritability, phenotypic and genetic correlation

A genomic relationship matrix between the animals was firstly generated with the *-grm* method in GCTA, and afterwards considered for the estimation of the genetic parameters [28, 74]. The restricted maximum likelihood (REML) method was applied for genetic parameter estimation. The respective genetic-statistical model was defined as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e} \tag{1}$$

where $\mathbf{y}$ was a vector of morphometric traits; $\mathbf{b}$ was a vector of fixed effects including age, sex, and AEZ_Breed; $\mathbf{u}$ was a vector of polygenic effects with a variance-covariance structure of $\mathbf{u} \sim N(0, \mathbf{G}\sigma_u^2)$, $\mathbf{G}$ was the genomic relationship matrix between individuals [74], σ_u^2 was the polygenic variance; $\mathbf{e}$ was a vector of random residual effects with $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$, $\mathbf{I}$ was an identity matrix of dimension $n \times n$ (with n, the sample size = 449); and $\mathbf{X}$ and $\mathbf{Z}$ were incidence matrices for $\mathbf{b}$ and $\mathbf{u}$, respectively.

Considering two conformation traits x and y, the genetic correlation (r_g) between x and y was estimated using the *"--reml-bivar x y"* option in bivariate genomic REML analyses. The phenotypic correlation (r_p) between x and y was calculated from the bivariate genomic REML outputs using the following formula:

$$r_{p_{xy}} = \frac{\sigma_{u_{xy}} + \sigma_{e_{xy}}}{\sqrt{(\sigma_{u_x}^2 + \sigma_{e_x}^2) \times (\sigma_{u_y}^2 + \sigma_{e_y}^2)}} \tag{2}$$

where $\sigma_{u_{xy}}$ and $\sigma_{e_{xy}}$ were the genetic covariance and residual covariance between x and y, respectively; and σ_u^2 and σ_e^2 were the genetic variance and residual variance of x and y, respectively. The standard errors of the phenotypic correlation were calculated based on the "delta-method" function from the R-package "msm" [147].

Multi-breed GWAS

GWAS were performed for the six morphometric traits using PLINK [27]. A linear regression using an additive genetic model was applied, and defined as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Wg} + \mathbf{e} \tag{3}$$

where $\mathbf{y}$ was a vector of morphometric traits; $\mathbf{b}$ was a vector of fixed effects including Age, Sex, AEZ_Breed, and linear discriminant functions; $\mathbf{g}$ was a vector for the SNP effects; $\mathbf{e}$ was a vector of random residual effects with $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$; and $\mathbf{X}$, $\mathbf{W}$ were incidence matrices for $\mathbf{b}$ and $\mathbf{g}$, respectively.

For a verification of results from PLINK, we additionally performed GWAS applying the following model (eq. 4) in the GCTA software [28]. However, considering the complexity of the mixed linear model in GCTA [28], and the small size of our dataset, only SNP detected by PLINK are prioritized and described. In matrix notation, the mixed model is:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Wg} + \mathbf{Zu} + \mathbf{e} \tag{4}$$

where $\mathbf{y}$, $\mathbf{g}$, $\mathbf{e}$ and incidence matrices $\mathbf{X}$ and $\mathbf{W}$ were notations as defined in Eq. 3; $\mathbf{b}$ was a vector of fixed effects including Age, Sex, AEZ_Breed; $\mathbf{Z}$ and $\mathbf{u}$ were notations as defined in Eq. 1.

The assessment of the models for population stratification based on the genomic inflation factor (lambda-λ) and on the quantile–quantile (Q–Q) plot. Manhattan and Q-Q-plots plots were generated by means of the ggplot2 package in R [148].

Significantly associated SNP were detected according to the Bonferroni corrected significance threshold ($p = 1.55 \times 10^{-06}$), i.e., calculated as $p = 0.05 / m$, with $m = 32,185$ (the effective number of SNP). In addition, chromosome-wide Bonferroni-corrected significance thresholds ($p_c = 0.05 / m_c$) with m_c denoting the effective number of SNP for each chromosome (see Additional file 5, Table S4) were used to identify suggestively associated variants. The effective numbers of SNP for the whole genome (m) and for each chromosome (m_c) were determined from the genetic type I error calculator (GEC) [149].

Candidate genes and functional annotations

To identify potential candidate genes associated with the morphometric traits, the rs-accession numbers of the significant and suggestive SNP were retrieved from the Ensembl genome database (version 96), using the BioMart R package [150, 151]. Genes were mapped to identified SNP, and only those located within a window frame of ±25 kb around each SNP were considered. In cases with more than two identified genes within the defined window frame, preference was given to the gene in which the SNP was located, or to the gene in closest SNP distance. However, if the SNP were located between two genes, both genes were selected, and the remaining genes were discarded. The gene mapping method as well as the window frame of ±25 kb were chosen to increase precision in selection of candidate genes.

Further, functional annotation was performed on the set of identified candidate genes per trait using the DAVID (see Additional file 6, Table S5 for the extensive outputs) [53]. In addition, pathways (KEGG and reactome pathways) and the biological process GO terms for candidate genes were retrieved manually, in order to infer potential gene functions.

Supplementary Information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12864-020-07170-0 .

Additional file 1: Figure S1. Scatterplots for the first four linear discriminant functions (LDF) included as covariates in GWAS model for population stratification in the four Beninese indigenous cattle breeds. The coloring represents the original breed assignment of samples.

Additional file 2: Table S1. Characteristics and origins of sampled animals in four Beninese indigenous cattle breeds. Animal ID, sex, breed, year of sampling and origin: farm longitude and latitude, commune, agro-ecological zones (AEZ).

Additional file 3: Figure S2. The variations of six morphometric traits from all (ALL) and from four respective Beninese indigenous cattle. Height at withers (HAW), sacrum height (SH), heart girth (HG), hip width (HW), body length (BL) and ear length (EL).

Additional file 4: Table S2. Effects of AEZ_B, sex and age in multi-factor linear models on six morphometric traits in four Beninese indigenous cattle breeds. Analysis of variance (ANOVA) table presenting the significance of fixed effects on height at withers (HAW), sacrum height (SH), heart girth (HG), hip width (HW), body length (BL) and ear length (EL). **Table S3.** Genome-wide and chromosome-wide significant SNP associated with six morphometric traits from GWAS model including the first four principal components (instead of the linear discriminant functions as considered for main results).

Additional file 5: Table S4. Chromosome-wide significance thresholds used in multi-breed GWAS for conformation traits in four Beninese indigenous cattle breeds.

Additional file 6: Table S5. Functional annotation of candidate genes for six morphometric traits in four Beninese indigenous cattle breeds. Results retrieved from the database for annotation, visualization and integrated discovery (DAVID) for candidate genes associated with height at withers (HAW), sacrum height (SH), heart girth (HG), hip width (HW), body length (BL) and ear length (EL).

Abbreviations

AEZ: Agro-ecological zone; ANOVA: Analysis of variance; BL: Body length; DAPC: Discriminant analysis of principal components; DAVID: Database for annotation, visualization and integrated discovery; EL: Ear length; FAO: Food and agriculture organization of the United Nations; *GCTA*: Genome-wide complex trait analysis; GEC: Genetic Type I error calculator; GWAS: Genome-wide association study; HAW: Height at withers; HG: Heart girth; HW: Hip width; kb: Kilo base pairs = 1000 base pairs; KEGG: Kyoto Encyclopedia of Genes and Genomes; LD: Linkage disequilibrium; LDF: Linear discriminant functions; Mb: Mega bases pairs = 1000 kb = 1 million base pairs; PCA: Principal component analysis; REML: Restricted maximum likelihood; r_g: Genetic correlations; r_p: Phenotypic correlation; SH: Sacrum height; SNP: Single nucleotide polymorphism

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Authors' contributions

SFV, CS, LHD and SK designed the study. SFV collected, analyzed the data and drafted the manuscript. CS, TY and KB supported in statistical analyses and manuscript preparation. SK and LHD reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All the data supporting the results of this article are presented within the article or in the additional files. The raw phenotypic and genotypic data are stored in the cloud of the University of Giessen (https://jlubox.uni-giessen.de) and can be provided by the corresponding author on reasonable request.

Ethics approval and consent to participate

The data were collected in accordance to the FAO guidelines for the characterization of animal genetic resources. Animal management, animal husbandry and trait recording followed the criteria for the assessment of animal welfare as identified and defined in by the Welfare Quality Project (WQP) [29, 152]. In Benin, there is no specific legislation for animal welfare or animal body measurements and hair sampling [153]. Hair sampling and body measurements are non-invasive methods. Hence, no approval was necessary. Before data collection, all farmers and cattle breeders gave their verbal consent through the Communal Sector for Agricultural Development (SCDA). The SCDA is the decentralized governmental institution for the management of the agricultural sector in the surveyed municipalities. In consequence, no further institutional approval was required.

Consent for publication

Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Publication 4. **First DNA Sequencing in Beninese Indigenous Cattle Breeds Captures New Milk Protein Variants**

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First DNA Sequencing in Beninese Indigenous Cattle Breeds Captures New Milk Protein Variants

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Abstract: This study investigated polymorphisms in the milk protein genes *CSN1S1*, *CSN2*, *CSN1S2*, *CSN3*, *LALBA*, and *LGB*, and casein haplotypes in Beninese indigenous cattle. Considering 67 animals, DNA sequencing of the genes' exons, flanking regions and parts of the 5′-upstream regions identified 1058 genetic variants including 731 previously unknown. In addition, four novel milk protein variants were detected, including $CSN3^{K}$ (p.Ala66Val), $LALBA^{F}$ (p.Arg58Trp), LGB^{B1} (p.Ala134Val) and LGB^{K} (p.Thr92Asnfs*13). $CSN3^{K}$ is caused by a novel SNP (BTA6:85656526C>T, exon 4) whereas $LALBA^{F}$ and LGB^{B1} are due to rs714688595C>T (exon 1) and rs109625649C>T (exon 4), respectively. Regarding LGB^{K}, a frameshift insertion of one adenine residue at BTA11:103257980 (exon 3) induces a premature translation termination resulting in a 46% reduction of the reference protein sequence. The casein polymorphisms formed five main *CSN1S1-CSN2-CSN1S2-CSN3* haplotypes including B-A1-A-B, B-A1-A-A and C-A2-A-B which are predominant in the investigated cattle breeds. Moreover, in silico analyses of polymorphisms within the 5′- and 3′- untranslated regions of all six milk proteins revealed effects on microRNA and transcription factor binding sites. This study suggests a large genetic variation of milk protein genes in Beninese cattle, which should be investigated in further studies for their effects on milk production, including quality and yield traits.

Keywords: casein and whey protein genes; genetic variants; casein haplotype; African taurine and indicine breeds; microRNA; transcription factor binding sites

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1. Introduction

The dairy sector in Benin, as in many African countries, is characterized by low productivity and strong dependence on imports to meet the growing demand for dairy products [1,2]. To address this situation, governments and farmers are opting for exogenous breeds, perceived as more productive. For example, the Girolando and Azawak breeds were introduced in Benin for crossbreeding in order to improve herd productivity [2,3]. Also, crossbreeding between local taurine and zebu breeds is increasingly practiced by small-scale farmers [4]. However, neither selection strategies nor proper documentation on the characteristics of the local breeds exist as a basis for optimized crossbreeding practices [5,6]. Imported exotic breeds are challenged due to their inferior adaptation to local harsh environments, implying increased disease frequencies and ongoing declines in productivity. A focus on exotic breeds also implies a growing risk to lose specific values and genetic diversity characterizing the local breeds [1,2]. Indeed, the taurine cattle (Somba, Lagune) are typically acknowledged for the appreciable organoleptic and cheesemaking qualities of their milk implying high economic values [7,8]. In contrast, the zebu cattle are known for higher milk yield, but of poorer milk quality [7]. As argued by Said Ahmed et al. [9], conservation and breeding strategies to improve milk yield should consider the maintenance of genetic diversity and conservation of specific traits of interest, including nutritional, immunological and technological quality of milk.

Six milk protein genes influence milk production traits. They include four casein genes (*CSN1S1*, *CSN2*, *CSN1S2*, *CSN3*) on *Bos taurus* autosome (BTA) 6 and two whey protein genes (*LALBA*/*LAA* and *LGB*/*PAEP*) on BTA5 and BTA11, respectively [10,11]. The genes encode the proteins α_{s1}-casein (CN), β-CN, α_{s2}-CN, κ-CN, α-Lactalbumin (α-LA), and β-Lactoglobulin (β-LG), respectively. The four caseins form a 250-kb cluster resulting in a haplotype on BTA6 [12]. Protein electrophoresis, mRNA- and DNA-sequence analyses identified enormous variability within the six main milk proteins [13,14]. The protein polymorphisms described in bovine milk include to date 10 protein variants for *CSN1S1* (A, B, C, D, E, F, G, H, I, J), 15 protein variants for *CSN2* (A1, A2, A3, B, C, D, E, F, G, H1, H2, I, J, K, L), five protein variants for *CSN1S2* (A, B, C, D, E), 14 protein variants for *CSN3* (A, A1, B, B2, C, D, E, F1, F2, G1, G2, H, I, J), five protein variants for *LALBA* (A, B, C, D, E), and 11 protein variants for *LGB* (A, B, C, D, E, F, G, H, I, J, W) [14–16]. Some protein variants, including the $CSN1S1^{H}$ and $CSN3^{J}$, are exclusively described in the West-African taurine breed Baoulé and the crossbreed Kuri, respectively [17]. Regarding the Beninese cattle breeds, only two studies addressed milk protein gene variability [18,19]. Both studies considered samples collected in 1997 and 1998, and described divergent distribution of milk protein variants in the breeds, especially between the taurine (Lagune and Somba) and the crossbreed (Borgou) [18,19]. An unknown variant α_{s1}-CN^{X} identified by Moazami-Goudarzi et al. [18] in Borgou animals is not clarified on DNA or mRNA level to date. Several investigations on cattle breeds in other countries associated milk protein variants with milk yield, composition and coagulation traits [20–22]. For instance, the β-$\mathrm{CN}^{\mathrm{A2}}$, predominant in zebu cattle presented positive effects on calf growth [23]. This variant is also of high interest for human health because of its ability to promote better digestion efficiency and antibacterial activity [14,17,24]. In contrast, the β-$\mathrm{CN}^{\mathrm{A1}}$ and κ-CN^{B} variants, observed in high frequency in the Beninese taurine breeds, have been linked to better cheesemaking properties (curd consistency, milk or rennet coagulation, micelle size). These descriptions match the appreciable features of Beninese taurine milk as described by the farmers [25,26]. Therefore, in Beninese cattle, we hypothesize a high correlation between milk production with milk protein genetic variability.

In addition to single milk protein variants, casein clusters significantly affected milk characteristics in ruminants [27]. Researchers reported significant relationships between different haplotypes and milk protein yield, coagulation, cheesemaking properties and offspring performances [28,29]. Braunschweig et al. [30] compared the parental haplotypes B-B-A-A and B-B-A-B for *CSN1S1-CSN2-CSN1S2-CSN3* in Swiss Brown Cattle and associated B-B-A-A with lower casein content and higher whey protein content in daughter milk. Similarly, the maternal composite casein genotype $CSN1S1^{\mathrm{BB}}$-$CSN2^{\mathrm{A2A2}}$-$CSN1S2^{\mathrm{AA}}$-$CSN3^{\mathrm{AB}}$ have been recently associated with pre-weaning offspring growth in German cattle [23].

Furthermore, polymorphisms occurring in the promoter regions of the milk protein genes are known to affect the binding sites of transcription factors (TF) or microRNA (miRNA) [31]. Transcription factors are proteins that bind to a specific DNA sequence (transcription factor binding sites, TFBS) in the cis-regulatory/intergenic regions to modulate the transcription or expression of a gene [32,33]. Similarly, miRNA, small non-coding RNA, interact with the 5′- and 3′-untranslated regions (UTR) and with gene promoter regions to suppress or activate expression, or control the translation and transcription rate of a gene [34–36]. Various single nucleotide polymorphisms (SNP) are located in TFBS or miRNA target sites flanking the milk protein genes, and are implicated in the alteration or reduction of milk protein gene functions (e.g., resulting in lower protein content) [9,37–39]. For example, the g.-475C>G SNP positioned within the activator protein-2 (AP-2) TFBS was associated with differential expression of LGB^{A} and LGB^{B} [40,41]. Likewise, SNP-haplotypes in the promoter regions of the *CSN1S1* are linked with different promoter alleles affecting milk production traits [42,43]. Hence, investigating the exons and flanking regions of milk protein genes in Beninese indigenous cattle breeds is beneficial to increase knowledge on existing genetic variation and on ongoing evolutionary patterns, to unravel their potential impacts in milk production, and to establish appropriate management strategies [9,44].

Screening milk protein polymorphisms and genotype composites and casein haplotypes is particularly important in Benin given the increasing crossbreeding and environmental pressures [45,46]. Caroli et al. [14] recommended frequent monitoring of milk protein genetic variability in cattle populations to detect and prevent the expansion of undesirable genetic mutations with unfavorable effects on milk traits. Gallinat et al. [15] recently identified new milk protein variants, reflecting the evolutionary pattern in different cattle breeds. With regard to the variety of tools to assess genetic polymorphisms in milk protein genes and haplotypes (at protein or DNA level), genomic methods provide a wider overview of chromosome variations [14,23]. The latter approach is increasingly being used to characterize known and novel polymorphisms in cattle milk protein genes [9,14,47]. Consequently, in the present study, we performed the first DNA sequencing of Beninese cattle, focusing on exons and flanking intron regions and parts of the 5′-upstream regions of milk protein genes, and we explored their functional consequences in silico. Milk protein polymorphisms and casein haplotypes were subsequently characterized in the Beninese indigenous cattle breeds.

2. Materials and Methods

2.1. Animal Sampling, DNA Extraction and Sequencing

Sixty-seven animals consisting of 20 Lagune, 20 Somba, 27 crossbreeds (including Borgou and Pabli animals) were selected within a large dataset of Beninese indigenous cattle sampled between 2016 and 2017 [45]. The Lagune and the Somba are two representatives of West African taurine, while the Borgou and Pabli are the products of crossbreeding, i.e., Somba × White Fulani (Zebu) and Somba × Borgou, respectively [6]. The relationships between the animals were minimized by considering only one sample per herd and per village. In addition, purebred Lagune and Somba animals with respective Lagune and Somba ancestry proportions $\geq$ 87% were selected, considering the values estimated with Admixture by Scheper et al. [45].

The DNA of the selected animals was extracted from hair samples using the NucleoSpin Tissue Kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany), following manufacturer's instructions. A minimum of 20 µL of DNA with a concentration of ~30 ng/µL were submitted to LGC Genomics GmbH (Berlin, Germany) for the sequencing of all exons and flanking intron regions as well as of parts of the 5′-regions of the casein and whey protein genes. The primer sequences described in Said Ahmed et al. [9] were used to amplify all exon sequences of the six milk protein genes under investigation. Moreover, additional primer pairs were established to amplify and subsequently sequence slightly larger 5′-upstream regions of the *CSN1S1*, *CSN3*, *LALBA* and *LGB* genes, respectively (see Table S1). Sequencing was made via Genotyping-in-Thousands by sequencing (GT-seq) using the Illumina MiSeq V3 platform with 2 × 300 bp paired-end read (LGC Genomics GmbH, Berlin, Germany). Using this method, next-generation sequencing of multiplexed polymerase chain reaction (PCR) products generated genotypes for a panel of DNA polymorphisms, in our case in the six main milk proteins in cattle.

2.2. Processing of Sequence Data

The raw sequence data consisting of 5 million reads were de-multiplexed with the Illumina bcl2fastq v2.20 [48]. Quality control using the FastQC v0.11.9 software filtered out low quality reads (containing ambiguous base, with final length inferior to 65 bases or with an average Phred quality score inferior to 30 over a window of ten bases). Subsequently, the BWA-MEM algorithm [49] was used to align the quality trimmed sequence reads against the respective reference sequences of cattle milk protein genes from the Ensembl genome database (http://www.ensembl.org/Bos_taurus/, accessed on 13 October 2021, see Table 1). Finally, variants and genotypes covered by a minimum coverage of 8 reads were called using the Bayesian haplotype-based method implemented in Freebayes v1.2.0, setting ploidy equal to 2. The outputs were checked and multiple nucleotide polymorphisms were manually processed to retrieve SNP, insertions and deletions (InDel).

Table 1. Reference gene sequences used to investigate milk protein gene polymorphisms in the Beninese indigenous cattle breeds.

Gene Name	**Gene Sequence**			
	Sequence ID	**Location**	**Strand** [1]	**Exons (*n*)**
CSN1S1	ENSBTAG00000007695	6:85410518:85429868	+	19
CSN2	ENSBTAG00000002632	6:85448564:85458344	−	9
CSN1S2	ENSBTAG00000005005	6:85529305:85549156	+	18
CSN3	ENSBTAG00000039787	6:85645254:85659526	+	5
LALBA	ENSBTAG00000005859	5:31182832:31213745	+	4
LGB	ENSBTAG00000014678	11:103255224:103264876	+	7

[1] + = forward strand, − = reverse strand.

2.3. Functional Annotation of DNA Sequence Variants, Frequencies of SNP Alleles and Casein Haplotypes

The positions of the identified sequence variants were inferred considering the gene sequence positions in the ARS-UCD1.2 genome assembly. Functional annotation, performed with the Ensembl Variant Effect Predictor online tool [50], retrieved known variants (referenced in the Ensembl database) and their potential consequence. The previously undescribed DNA variants were submitted to the European Variation Archive (EVA) which assigned individual variant accessions (ss#) number to them [51]. To avoid redundancies and discrepancies in the functional annotation of the polymorphisms, only the most conserved transcript was investigated for each milk protein gene (i.e., ENSBTAT00000010119.3 for *CSN1S1*, ENSBTAT00000003409.6 for *CSN2*, ENSBTAT00000006590.6 for *CSN1S2*, ENSBTAT00000028685.5 for *CSN3*, ENSBTAT00000007701.2 for *LALBA* and ENSBTAT00000019538.6 for *LGB*). Variants detected in the coding regions were considered to annotate milk protein variants following the existing nomenclature [9,14,15,52] and referring to the amino acid (AA) position in the mature proteins.

SNP allele frequencies in the respective breeds were calculated with the vcftools v0.1.16 software [53]. In addition, haplotypes were constructed across the casein cluster *CSN1S1-CSN2-CSN1S2-CSN3*, and their frequencies were estimated using the haplo.group function in the haplo.stats (version 1.8.7) R package [54]. In this regard, we estimated the maximum likelihood of haplotype probabilities in the different breeds as well as for the whole population. Only the casein variants with a minimum allele frequency of 5% in the overall population were considered for haplotype constructions.

Potential effects of the splice site variants were investigated in silico using the NNSPLICE 0.9 tool [55]. In addition, the polymorphisms located in the 5′- and 3′-UTR of the milk protein genes were examined for the prediction of TFBS and miRNA targets. TFBS were identified applying the sequence analysis workflow with the Gene Transcription Regulation Database (GTRD) [56,57] in the geneXplain platform 6.4 web server (https://platform.genexplain.com/bioumlweb/, accessed on 13 October 2021). For the investigation of miRNA target sites, the seed regions of bovine miRNA were downloaded on the TargetScan website (http://www.targetscan.org, accessed on 13 October 2021, see Table S2) and used applying the "targetscan_60.pl" algorithm [58,59].

3. Results

3.1. Distribution of DNA Sequence Variants

Sequencing of all exon sequences, flanking intron regions and parts of the 5′-upstream regions of the six milk protein genes, and subsequent comparisons with the Ensembl bovine reference sequence (Table 1), displayed a total of 987 SNP and 71 InDel. With regard to the total number of variants identified in the respective gene, the *CSN1S1*, *CSN1S2* and *LALBA* presented the highest proportions of InDel (Table 2). Only 30.91% (327 variants) of the detected polymorphisms were referenced in the Ensembl variant table, whereas 731 variants have not been reported before (Table S3). The latter variants include 21 SNP occurring at the same position of a known SNP (in the Ensembl variant table), but with new alleles (Table S3). In comparison to the other genes, *CSN3* displayed the highest proportion (88.85%) of newly identified variants (Figure 1). In contrast, the majority of the identified variants in *LALBA* and *LGB* are already referenced in the Ensembl variant table.

Table 2. Number and percentage distribution (in brackets) of the sequence variants (SNP, InDel) detected within the exon sequences, flanking intron sequences and parts of the 5′-flanking region of the six milk protein genes (*CSN1S1*, *CSN2*, *CSN1S2*, *CSN3*, *LALBA*, *LGB*) in Beninese indigenous cattle breeds.

	CSN1S1	***CSN2***	***CSN1S2***	***CSN3***	***LALBA***	***LGB***
			***n* (%)**			
SNP	20 (8.06)	8 (5.83)	26 (7.53)	15 (5.23)	3 (11.54)	1 (2.22)
InDel [1]	228 (91.94)	114 (94.17)	313 (92.47)	275 (94.77)	23 (88.46)	44 (97.78)
Total	248	120	332	287	26	45

[1] InDel = insertions/deletions.

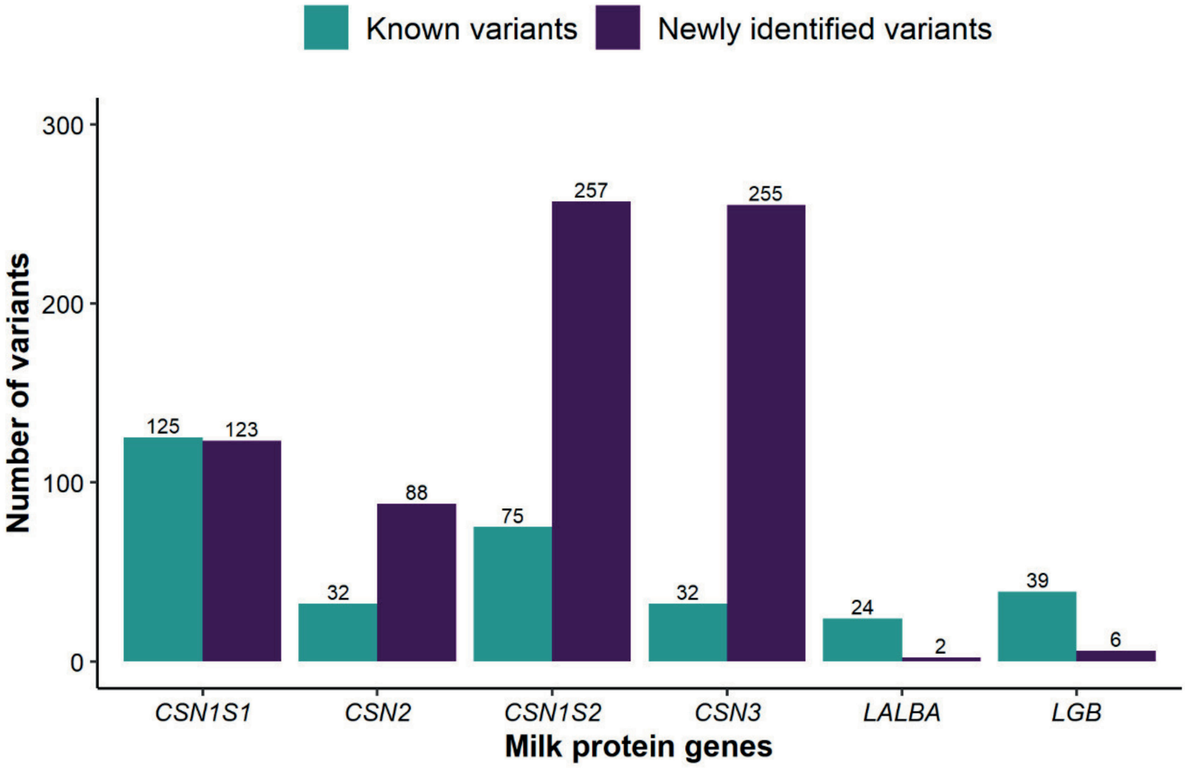

Figure 1. Distribution of known and novel DNA variants within the exon sequences, flanking intron sequences and parts of the 5′-upstream regions of milk protein genes (*CSN1S1*, *CSN2*, *CSN1S2*, *CSN3*, *LALBA*, *LGB*) in Beninese indigenous cattle breeds.

The variants were mainly detected in the intron regions (90.5%), 5′-UTR (2.3%), 5′-upstream (2%) and 3′-downstream (1.9%) regions (Figure 2). Only 2% of the detected variants were positioned in coding regions (missense, synonymous and frameshift variants). The *CSN3* and *LALBA* genes displayed the majority of the missense variants (three SNP for each of the genes). A frameshift deletion and insertion were identified in *LALBA* and *LGB*, respectively. In addition, a total of six synonymous SNP were detected in *CSN2*, *CSN3*, *LALBA* and *LGB* (Table S4).

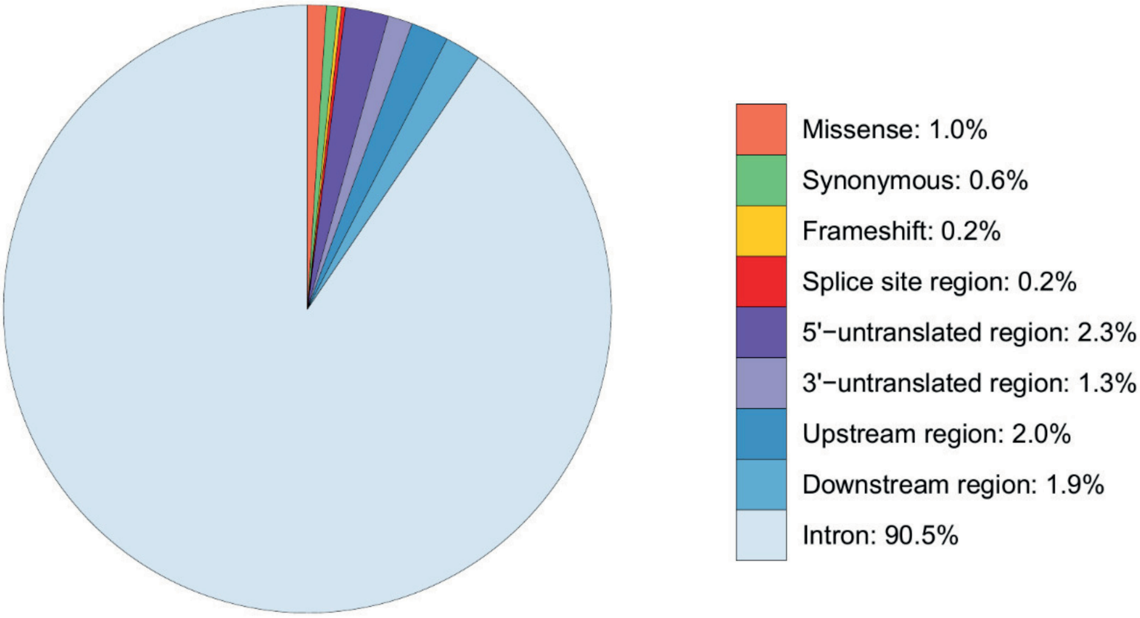

Figure 2. Overall percentage of the DNA variant types detected within the exon sequences (missense, synonymous, frameshift), flanking intron sequences and parts of the 5′-upstream regions of the six milk protein genes (*CSN1S1*, *CSN2*, *CSN1S2*, *CSN3*, *LALBA*, *LGB*) in Beninese indigenous cattle breeds. # The respective distributions of variant types for each gene (see above Figure 1) are presented in Table S4.

3.2. Causal Genetic Polymorphisms of Milk Protein Variants

A total of 11 missense SNP and one frameshift insertion have been identified in the milk protein genes in the investigated population. Table 3 presents the identified sequence differences, their locations within the respective gene, their effects on the protein sequence, the protein variant designations and frequencies within the different breeds and across all Beninese cattle. More detailed descriptions of the sequence differences found in the respective genes are given in Section 3.2.1 and in the following.

Table 3. Description (ID, location and allele frequencies) of the variants causing protein polymorphisms of the six milk protein genes (*CSN1S1*, *CSN2*, *CSN1S2*, *CSN3*, *LALBA*, *LGB*), their effects on the protein sequence and the designations of consequent protein variants, and frequencies within the different Beninese local breeds and across all investigated cattle.

Genes	**SNP**				**Gene Transcript**			**Variant Frequency**			
	ID [1]	**BTA:bp [2]**	**Allele**	**Exon**	**Protein [3]**	**Amino Acid [4]**	**Variant [5]**	**Crossbreed (*n* = 27)**	**Lagune (*n* = 20)**	**Somba (*n* = 20)**	**Total (*n* = 20)**
CSN1S1	rs43703010	6:85427427	A G	17	192 (207)	Glu Gly	B C	0.62 0.38	0.97 0.03	0.93 0.08	0.81 0.19
CSN2	rs715383373	6:85450908	T C	7	197 (247)	Val Ala	A1 L	0.97 0.03	1.00 0.00	1.00 0.00	0.99 0.01
	rs43703011	6:85451298	A C	7	67 (117)	His Pro	A1 A2	0.39 0.61	0.80 0.20	0.63 0.37	0.57 0.43
CSN1S2	rs441966828	6:85533780	C T	3	8 (23)	Ser Phe	A B	0.98 0.02	1.00 0.00	1.00 0.00	0.99 0.01
CSN3	ss7626433428	6:85656526	C T	4	66 (87)	Ala Val	B K	1.00 0.00	0.75 0.25	1.00 0.00	0.97 0.03
	rs43703015	6:85656736	T C	4	136 (157)	Ile Thr	B A	0.50 0.50	0.75 0.25	0.80 0.20	0.72 0.28
	rs43703016	6:85656772	C A	4	148 (169)	Ala Asp	B A	0.50 0.50	0.50 0.50	0.80 0.20	0.69 0.31

Table 3. *Cont.*

Genes	SNP				Gene Transcript			Variant Frequency			
	ID [1]	BTA:bp [2]	Allele	Exon	Protein [3]	Amino Acid [4]	Variant [5]	Crossbreed (n = 27)	Lagune (n = 20)	Somba (n = 20)	Total (n = 20)
LALBA	rs714688595	5:31184282	C	1	10 (58)	Arg	B	0.96	1.00	0.88	0.94
			T			Trp	F	0.04	0.00	0.13	0.06
	rs722550244	5:31184283	G	1	10 (58)	Arg	B	0.88	1.00	1.00	0.95
			A			Gln	A	0.12	0.00	0.00	0.05
	rs465119286	5:31184696	A	2	41(89)	Ile	B	0.88	1.00	0.95	0.93
			G			Val	E	0.12	0.00	0.05	0.07
LGB	rs109625649	11:103259232	C	4	118 (134)	Ala	B	0.89	0.50	0.81	0.80
			T			Val	B1	0.11	0.50	0.19	0.20
	ss7626433430	11:103257980	-	3	Thr92	-	B	0.96	1.00	1.00	0.98
			A		Thr92Asnfs*13	-	K	0.04	0.00	0.00	0.02

[1] Variant ID: the rs# accession number of known variants were retrieved from the Ensembl database; the ss# accession number of the new variants identified from our sequencing were assigned by the European Variation Archive; [2] bp = SNP position according to the ARS-UCD1.2 genome map; [3] Amino acid (AA) position in mature protein. The corresponding AA positions in the protein sequence including signal peptide are given in parentheses; [4] Ala: Alanine, Arg: arginine, Asp: Aspartic acid, Gln: glutamine, Glu: glutamic acid, Gly: Glycine, His: histidine, Ile: isoleucine, Phe: Phenylalanine, Pro: proline, Ser: serine, Thr: Threonine, Trp: Tryptophan, Val: Valine; [5] Milk protein variants were inferred following previous nomenclature [9,14,15,52]. The underlined variants have been newly annotated.

3.2.1. CSN1S1

The sequencing of the 19 exons of the *CSN1S1* gene identified the already known casein variants $CSN1S1^{B}$ and $CSN1S1^{C}$. The latter protein variant is due to the SNP rs43703010C>T located in exon 17, inducing the substitution of the glutamic AA by glycine (p.Glu192Gly) in the mature protein. In our animal material, 81% of all animals, especially the taurine, had the reference allele rs43703010A, leading to $CSN1S1^{B}$. In contrast, the alternative allele rs43703010G, causing $CSN1S1^{C}$, was mainly present in the crossbreed animals with an intra-breed frequency of 38% (Table 3).

3.2.2. CSN2

We detected the casein variants $CSN2^{A1}$, $CSN2^{A2}$ and $CSN2^{L}$. $CSN2^{A2}$ is caused by rs43703011A>C in exon 7 (SNP), inducing the replacement of histidine by proline at position 67 of the mature protein (p.His67Pro, Table 3). The allele rs43703011C was dominant in the crossbreeds (intra-breed frequency of 61%). In contrast, the reference allele rs43703011A, leading to the casein variant $CSN2^{A1}$, was prevalent in the taurine, especially for Lagune (intra-breed frequency equal to 80%). The casein variant $CSN2^{L}$ is characterized by a replacement of valine by alanine (p.Val197Ala), due to the SNP rs715383373T>C in exon 7. The allele rs715383373C was identified in one crossbreed animal. Additionally, we identified the SNP rs468218273C>T affecting the AA 42 (p.Ala42) of the immature protein, located within the signal peptide.

3.2.3. CSN1S2

Only the SNP rs441966828C>T (exon 3) differing the protein variants $CSN1S2^{\mathrm{A}}$ and $CSN1S2^{B}$ was found in the investigated Beninese cattle population. Indeed, p.Ser8Phe is the resulting AA exchange differentiating $CSN1S2^{B}$ from $CSN1S2^{A}$. The reference SNP allele rs441966828C encoding for $CSN1S2^{A}$ was observed in the majority (99%) of the animals, while the allele rs441966828T producing the protein variant $CSN1S2^{B}$ was detected in one crossbreed animal (Table 3).

3.2.4. CSN3

The analysis of the five exons of *CSN3* detected two known ($CSN3^{A}$, $CSN3^{B}$) and one new casein variant ($CSN3^{K}$). The casein variants $CSN3^{A}$ and $CSN3^{B}$ are caused by the SNP rs43703015T>C and rs43703016C>A in exon 4, inducing the AA replacements p.Ile136Thr and p.Ala148Asp, respectively. Most of the animals carried the reference SNP alleles rs43703015T and rs43703016C, coding for $CSN3^{B}$ (Table 3). In contrast, the respective alleles rs43703015C and rs43703016A, coding for $CSN3^{A}$, were mainly observed in heterozygous genotypes in the crossbreed animals (50%). Additionally, we detected the synonymous

SNP rs110014544G>A (p.Ala168) also differentiating the casein variants *CSN3*^{A} and *CSN3*^{B}. The novel kappa-casein variant (*CSN3*^{K}) detected in this study is due to a previously undescribed SNP (BTA6:85656526C>T) in exon 4 leading to an AA exchange from alanine to valine (p.Ala66Val). The new variant *CSN3*^{K} was observed in one crossbreed animal.

3.2.5. LALBA

Across the four exons of *LALBA*, three missense SNP causing four milk protein variants including three known (*LALBA*^{A}, *LALBA*^{B}, *LALBA*^{E}) and one previously undescribed (*LALBA*^{F}), were identified. The SNP rs722550244G>C in exon 1 is responsible for the change of arginine to glutamine (p.Arg10Gln), differentiating *LALBA*^{B} from *LALBA*^{A}. The reference SNP allele rs722550244G causing *LALBA*^{B} was present in most of the animals (95%), whereas the alternative SNP allele rs722550244A leading to *LALBA*^{A} was only observed in crossbreed animals (Table 3). The protein variant *LALBA*^{E} is caused by an A to G nucleotide transition in exon 2 (SNP rs465119286A>G). The SNP allele rs465119286G found in 7% of the animals (including Somba and crossbreed) causes the replacement of isoleucine to valine (p.Ile41Val). In addition to the SNP rs722550244G>C that distinguishes *LALBA*^{B} from *LALBA*^{A}, the SNP rs7144688595C>T also affects the AA 10 of the mature protein α-LA. Here, the SNP leads to the AA exchange p.Arg10Trp. Therefore, we have introduced a new naming *LALBA*^{F} for this protein variant. The SNP allele rs7144688595T was observed in 6% of the animals. Moreover, a synonymous SNP rs477959124C>T (p.Asp64) was identified in exon 2.

3.2.6. LGB

Concerning the *LGB* gene, we identified three milk protein variants (*LGB*^{B}, *LGB*^{B1}, *LGB*^{K}) but only *LGB*^{B}, being the reference allele (Table 1) was previously described. Indeed, only one causal genetic polymorphism, i.e., the missense SNP rs109625649C>T differing the protein variants *LGB*^{B} and *LGB*^{A}, was detected in exon 4 (Table 3). The SNP allele rs109625649T observed in 20% of the animals induces an exchange of alanine to valine (p.Ala118Val). With the absence of the second missense SNP characterizing *LGB*^{A}, we considered the observed sequence as an intermediate and suggested a new variant name *LGB*^{B1}. The second novel protein variant *LGB*^{K} is caused by a frameshift insertion of an adenine nucleotide at position BTA11:103257980 in exon 3. This new polymorphism was detected in one animal and induced the emergence of a premature stop codon, reducing the coding sequence of the *LGB* gene from a total of 178 to 104 AA with a complete exchange of the protein sequence from AA 92 to 104 (p.Thr92Asnfs*13, see Table S5). Additionally, three synonymous SNP were mapped in the *LGB*, including rs109116595T>C (p.Ile2) in exon 1, rs110641366T>C (p.Asn88) and rs715512468C>T (p.Thr97) in exon 4.

3.3. Casein Haplotypes

A total of 24 casein haplotypes (*CSN1S1-CSN2-CSN1S2-CSN3*) were built from the identified casein variants (with individual allele frequency $\geq$ 5%). However, only five haplotypes display a frequency of 5% in at least one breed (Table 4). The haplotype B-A1-A-B was the most frequent, especially in Somba (47%) and Lagune (50%). In contrast, the haplotypes B-A1-A-A and C-A2-A-B were predominant in the crossbreeds. Moreover, the B-A2-A-B haplotype was observed in all breeds with comparable frequency.

Table 4. Frequencies of the bovine casein haplotypes (*CSN1S1*-*CSN2*-*CSN1S2*-*CSN3*) in the different Beninese breeds and across all investigated cattle.

Haplotypes [1]	Crossbreed (n = 27)	Lagune (n = 20)	Somba (n = 20)	Total (n = 20)
B-A1-A-B	0.01	0.50	0.47	0.39
B-A2-A-B	0.20	0.18	0.25	0.22
B-A1-A-A	0.39	0.29	0.20	0.20
C-A2-A-A	<0.01	<0.01	0.02	0.19
C-A2-A-B	0.38	<0.01	0.02	<0.01

[1] Only haplotypes with a minimum frequency of 5% in one breed are considered.

3.4. Functional Effects of Variants in the Splice Sites, 5′- and 3′-Untranslated Regions of Milk Protein Genes

In silico evaluations of the polymorphisms in the non-coding exonic regions of the six milk protein genes displayed various potential effects. The SNP rs452830840C>T and rs208412793G>T are positioned in the splice site regions of *CSN3* and *LGB*, respectively. However, they presented no impact on the binding score of the splice enzymes using NNSPLICE 0.9. Furthermore, no further identified SNP or InDel affected the 5′-donor or 3′-acceptor splice sites of the exons of the six genes.

The *CSN1S1* and *LALBA* genes presented several SNP in the 5′- and 3′-UTR and many of them influenced TFBS or miRNA target sites (Table 5). In contrast, we mapped only one SNP (rs440770944C>A) in the 3′-UTR of *CSN2*. This SNP altered the binding site of the bta-miR-2464-3p miRNA, but favored the p63 TFBS. Overall, 11 and 15 SNP located in the 5′-UTR of the six milk protein genes affected TFBS or miRNA target sites, respectively. Six SNP were responsible for the loss of TFBS, whereas seven SNP promoted the gain of TFBS. They included one 5′-UTR SNP (rs109900747T>C) causing the loss and the gain of TFBS simultaneously. Five SNP abrogated miRNA target sites in the 5′-UTR, while five other SNP introduced new miRNA target sites. In addition, simultaneous loss and gain of miRNA target sites were observed in five SNP located in the 5′-UTR. Within the 3′-UTR of the milk protein genes, five and 10 SNP altered TFBS and miRNA target sites, respectively. In contrast, two and five SNP introduced new TFBS and miRNA target sites, respectively.

Table 5. Potential effects of SNP positioned within the 5′- and 3′-untranslated regions (UTR) of bovine milk protein genes on the binding of micro-RNA (miRNA) and transcription factors (TF) in Beninese cattle breeds.

Gene	Location (BTA:bp [1])	Allele (ref/alt) [2]	ID [3]	Position	miRNA		TF	
					Loss [4]	Gain [4]	Loss [4]	Gain [4]
CSN1S1	6:85411136	C/T	rs379920406	5′-UTR	-	bta-miR-2291	-	-
	6:85411147	G/A	rs517257790	5′-UTR	-	-	-	-
	6:85411192	C/T	rs520777414	5′-UTR	-	-	-	-
	6:85411221	A/G	rs133040184	5′-UTR	bta-miR-133c	bta-miR-9-5p	-	-
	6:85411236	A/G	rs137119956	5′-UTR	bta-miR-2446	-	E2F-6	-
	6:85411427	A/C	rs135615686	5′-UTR	-	-	RAR	-
	6:85411578	A/G	rs109817504	5′-UTR	-	-	ATF-3; B-ATF; c-Jun; SMARCC1; SMARCC2	-
	6:85411618	C/G	rs109757609	5′-UTR	bta-miR-2284e	bta-miR-7864	-	Sp1; IRF-4
	6:85411677	G/A	rs134534951	5′ UTR	-	-	STAT5A	-
	6:85411780	T/C	rs110163054	5′-UTR	bta-miR-15a; bta-miR-562	-	-	-
	6:85413195	C/T	rs110899610	5′-UTR	bta-miR-2325b; bta-miR-2420; bta-miR-453	bta-miR-376a	-	-
	6:85428962	G/A	rs466704456	3′-UTR	bta-miR-874	bta-miR-219-5p; bta-miR-2355-3p	-	-
	6:85429024	C/T	ss7626432754	3′-UTR	-	-	-	-
	6:85429077	A/G	rs435231328	3′-UTR	bta-miR-338	-	-	-
	6:85429241	A/G	rs211141048	3′-UTR	bta-miR-380-3p	-	SMAD4	-
	6:85429252	C/A	rs716970086	3′-UTR	bta-miR-371	-	SMAD4	-
CSN2	6:85449252	C/A	rs440770944	3′-UTR	bta-miR-2464-3p	-	-	p63

Table 5. *Cont.*

Gene	Location (BTA:bp [1])	Allele (ref/alt) [2]	ID [3]	Position	miRNA		TF	
					Loss [4]	Gain [4]	Loss [4]	Gain [4]
CSN1S2	6:85531897	T/C	rs109900747	5′-UTR	-	bta-miR-452	Sp1; HNF-4alpha; HNF-4gamma; FOXO1	c-Ets-1
	6:85548443	A/G	rs211156498	3′-UTR	bta-miR-2318; bta-miR-2479; bta-miR-2480	bta-miR-2463; bta-miR-452	CP2-L1	-
	6:85548520	A/G	rs109274107	3′-UTR	-	bta-miR-1388-3p	-	-
CSN3	6:85656841	T/A	rs109787476	3′-UTR	bta-miR-496	bta-miR-2284w	ZNF274	
	6:85658779	C/A	ss7626433382	3′-UTR	bta-miR-193a-3p	-	-	-
	6:85658873	G/A	rs134516686	3′-UTR	-	-	-	-
LALBA	5:31183709	G/C	rs444727593	5′-UTR	-	bta-miR-2475	-	-
	5:31183736	G/A	rs458466372	5′-UTR	bta-miR-2284r; bta-miR-2284s	-	-	-
	5:31183766	T/C	rs471361585	5′-UTR	-	bta-miR-2370-5p	-	-
	5:31183789	A/G	rs440012037	5′-UTR	bta-miR-7864		-	-
	5:31183806	C/T	rs717249686	5′-UTR	-	-	-	KLF4
	5:31183848	C/A	rs460157851	5′-UTR	-	bta-miR-2472; bta-miR-6517	-	AP-2alpha; IRF-9, STAT2;STAT1
	5:31183921	A/G	rs448925171	5′-UTR	bta-miR-2325c; bta-miR-6120-3p	bta-miR-218; bta-miR-2452	-	-
	5:31183924	T/C	rs719631407	5′-UTR	bta-miR-6120-3p	bta-miR-218	-	-
	5:31184082	T/C	rs462561324	5′-UTR	-	-	-	-
	5:31185981	C/T	rs718675014	3′-UTR	-	bta-miR-2425-5p; bta-miR-2467-3p	N-Myc; N-Myc	c-Myc
	5:31186027	A/G	rs110359174	3′-UTR	bta-miR-143	-	-	-
LGB	11:103255847	G/A	rs516356159	5′-UTR	-	-	-	RCoR2
	11:103255918	C/T	rs41255682	5′-UTR	bta-miR-1777a; bta-miR-2454-5p	-	SIX5	-
	11:103255930	G/C	rs41255683	5′-UTR	-	-	-	STAT1; STAT3
	11:103260824	G/A	rs1116405113	3′-UTR	bta-miR-7860	-	-	-

[1] bp = SNP position; [2] ref/alt = reference/alternate allele, [3] Variant ID: the rs# accession number of known variants were retrieved from the Ensembl database; the ss# accession number of the new variants identified from our sequencing were assigned by the European Variation Archive; [4] loss of binding, gain of binding.

4. Discussion

In this study, we sequenced the exons, flanking intron regions, and parts of the 5′-flanking regions of the six milk protein genes from 67 animals, and identified a total of 1058 DNA polymorphisms. This value is higher than those reported in previous studies targeting sequence data of cattle milk protein genes [9,60]. For instance, Meier et al. [60] considered the 1000 Bull Genomes with 1821 animals from 14 different breeds and reported only 892 SNPs within the four casein genes (exons and flanking intron regions) and their 1000 bp upstream regions. In contrast, the four casein genes display 987 variants in our study. This difference implies the high level of polymorphisms in the milk protein gene segments of the Beninese breeds compared to other cattle breeds. In addition, the large number of novel variants is typical in the context of the pronounced genetic diversity of the Beninese cattle, and concurs with the need for deeper investigation of African livestock genomes [45,61].

Similar to Meier et al. [60], we observed the majority of the polymorphisms in the intron regions. However, several variants are also located in the coding regions as well as in the 5′- and 3′-UTR. We only focus on the polymorphisms in 5′- and 3′-UTR for the screening of TFBS or target sites for miRNA because of the large number of detected SNP. The identified effects of the 5′- and 3′-UTR SNP on TFBS and miRNA sites (see Table 5) are consistent with previous findings and suggest further investigations on the SNP located in the 5′-upstream and 3′-downstream region of the milk protein genes [9,38]. The analyses of the SNP impacts on the splice site recognition of the milk protein genes using NNSPLICE revealed no effect. However, supplementary tools (e.g., ESEfinder) can be applied to investigate exonic splicing enhancers in the milk protein gene [62]. Moreover, further research is needed to clarify the real effects of these polymorphisms in the TFBS, miRNA binding sites and splice site regions, for example using PCR-based expression studies (like real-time quantitative PCR or droplet digital PCR), gene expression array or RNA sequencing (RNA-Seq) methods [63–66].

Within the coding regions, a total of 12 DNA polymorphisms caused milk protein variants. They include two previously unknown DNA variants, i.e., one SNP in *CSN3* and one frameshift insertion in *LGB*. The total number of missense variants is appreciable, but less than those (a total of 20 missense SNP in casein genes) reported in the 1000 Bull Genomes consisting of a large variety of cattle breeds [60]. Nevertheless, the higher number of missense variants identified in *CSN2*, *CSN3* and *LALBA* in the present study are correlated with the high polymorphisms of these milk protein genes compared to the others [14,60]. Several other segregating variants of the bovine milk protein genes were also not identified [14,15]. Overall, 17 milk protein variants were characterized and included two for *CSN1S1* (B, C), three for *CSN2* (A1, A2, L), two for *CSN1S2* (A, B), three for *CSN3* (A, B, K), four for *LALBA* (A, B, E, F), and three for *LGB* (B, B1, K). $CSN3^K$, $LALBA^F$, LGB^{B1} and LGB^K are undescribed so far.

4.1. Sequence Polymorphisms and Milk Proteins in Beninese Cattle

4.1.1. CSN1S1

The detection of $CSN1S1^B$ and $CSN1S1^C$ substantiates the previous identification of α_{s1}-CN^B and α_{s1}-CN^C at protein level in Beninese cattle [18]. The lower distribution of the protein variant $CSN1S1^C$ in the taurine breeds (in contrast to the crossbreeds) is consistent with results in other African cattle breeds [9,17,19]. The association of the $CSN1S1^C$ variant with impaired immunoreactivity in humans (in contrast to $CSN1S1^B$) suggests its conservation through the valorization of milk from zebu and crossbreed animals for human health [67]. We are not able to elucidate the rare allele α_{s1}-CN^X identified by Moazami-Goudarzi et al. [18] by protein electrophoresis in the Borgou cattle. Therefore, analyses on protein level using milk samples is necessary to infer the protein allele α_{s1}-CN^X and to clarify its DNA sequence difference. The isoelectric focusing method described by Giambra et al. [68] with adjacent mRNA or DNA sequencing can be a useful approach in this regard.

Seven SNP within the 5′-UTR of *CSN1S1* represent the causal polymorphisms of the *CSN1S1* promoter alleles 1, 2, 3 and 5, as previously described by Prinzenberg et al. [43] and Ibeagha-Awemu et al. [42]. However, only the *CSN1S1*Prom^1 and *CSN1S1*Prom^2 were fully identified in our samples. The *CSN1S1*Prom^3 and *CSN1S1*Prom^5 diverge from our sequences with the absence of some characteristic polymorphisms.

Moreover, we observed various effects of the SNP located in the 5′- and 3′-UTR of the *CSN1S1* gene on TFBS and miRNA targets. Similar impacts of SNP in the *CSN1S1* flanking regions on TFBS and milk protein content have been previously reported in cattle [42,43] and in small ruminants [69].

4.1.2. CSN2

The distributions of $CSN2^{A1}$ and $CSN2^{A2}$ in the Somba and crossbreed populations are in line with previous findings [18,19]. Some differences in the allele frequencies of the two alleles may be due to a greater local limitation in the samples of Moazami et al. [18]. In contrast to our study, the authors only studied Lagune and Borgou animals from the regions of Porto Novo and Borgou, respectively. Due to a wider distribution of the animals sampled in our study in Benin and an associated low relationship between the animals, it is possible that we were able to present the more current allele frequency for these casein variants in Beninese cattle. However, we did not detect the variant $CSN2^I$ reported by Ceriotti et al. [19] in the Borgou. Interestingly, we found $CSN2^L$ in a crossbreed animal. The protein variant has recently been described at low frequency (0.02) in Gyr cattle by Gallinat et al. [15]. Said Ahmed et al. [9] similarly observed this protein variant in 10% of their genotyped Butana cattle. However, recent analysis of the 1000 Bull Genomes considering 14 different taurine breeds did not detect $CSN2^L$. In consequence, our findings reinforce the specificity of $CSN2^L$ to indicine cattle. Further rare variants $CSN2^B$ and $CSN2^D$ previously reported in other African taurine (Baoulé, N'Dama) and indicine breeds (Sudanese Fulani, zebu Shuwas Arab) [17] were not identified in our study.

In contrast to the other investigated milk protein genes, we detected no SNP in the 5′-UTR and only one SNP in the 3′-UTR of *CSN2*. The sequence analyses suggested that the latter SNP (rs440770944C>A) induces the binding of the p63 TF. However, no influence of the p63 TF on milk protein gene transcription has been previously reported. In addition, the impact of SNP positioned in non-coding regions of the *CSN2* gene has been rarely reported in cattle. Only one SNP located in the 5′-upstream region (g.1311T>C) of the caprine *CSN2* was associated with a lack of β-CN in milk [70].

4.1.3. CSN1S2

In contrast to the other milk protein genes, no previous study reported the distribution of the protein variants of *CSN1S2* in the Beninese cattle breeds. The gene has a low polymorphic level in cattle, and consequently, it was only marginally addressed [71]. The detection of the $CSN1S2^A$ in 99% of the investigated animals confirm its prevalence in cattle [14]. In addition, the protein variant $CSN1S2^B$ is detected in only one crossbreed animal, in agreement with its specificity to indicine cattle [71]. Ibeagha-Awemu et al. [71] observed this variant in European cattle breeds influenced by zebu introgression. In addition, $CSN1S2^D$ and $CSN1S2^E$ reported in low frequencies in some European and Iranian cattle breeds, respectively, were not found in this study [14,15,71].

Only one SNP (rs109900747T>C) was observed in the 5′-UTR of *CSN1S2*, whereas Said Ahmed et al. [9] reported two other SNP in this region. The alternative allele rs109900747C alters several TFBS, but promotes the c-Ets-1 TFBS and the target site of the bta-miR-452 miRNA. Similar results were reported by Kishore et al. [39] in the 5′-flanking region of *CSN1S2* in Indian zebu cattle. Furthermore, two SNP in the 3′-UTR induce the deletion and/or the substitution of several miRNA binding sites. Some of these miRNA were associated with milk production traits including milk fat metabolism [72,73]. Therefore, the impact of the alteration/substitution of miRNA target sites from SNP in 3′-UTR of *CSN1S2* would be interesting for investigations in an experimental study.

4.1.4. CSN3

The identification of $CSN3^A$ and $CSN3^B$ in Beninese cattle breeds is in accordance with previous results [18,19]. In addition, the high frequencies of the $CSN3^B$ causative SNP supports the known prevalence of this variant in African taurine [14,17]. Further variants $CSN3^{A1}$, $CSN3^H$ and $CSN3^J$ as previously reported in African indicine and taurine breeds, respectively, were not detected in our samples. However, the SNP (BTA6:85656526C>T) causing p.Ala66Val is interesting, as it has never been reported before. The *CSN3* is highly polymorphic with several variants (e.g., A^1, E, F^2, H, I) due to only one SNP, respectively [14]. We name this new variant $CSN3^K$ following the previous nomenclature in cattle [13,14].

We identified a SNP (BTA6:85658779C>A) in the 3′-UTR of *CSN3*, which has not been described before. This SNP abrogates the target site of the bta-miR-193a-3p. Additionally, the 3′-UTR of *CSN3* displays two other SNP including the rs109787476T>A, which affect miRNA target sites. Said Ahmed et al. [9] similarly reported several SNP affecting miRNA binding in this region in the Butana cattle, but none of these SNP was identified in our study. Moreover, a SNP in the non-coding region of the *CSN3* gene was associated with milk production traits [14,74]. In consequence, the detected polymorphisms in the 3′-UTR of *CSN3* are of interest as they may affect the expression of the gene, and consequently, they influence the technological quality of milk in Beninese cattle. Indeed, the importance of the *CSN3* gene in cheesemaking properties of milk is well established [14].

4.1.5. LALBA

In *LALBA*, we observed the predominance of the variant $LALBA^B$, while $LALBA^A$ was only found in some crossbreed animals. These findings are in agreement with reports addressing Beninese cattle as well as other African cattle breeds [9,14,17,18]. No taurine animal presents the $LALBA^{A}$ variant in our samples. This variant is more prevalent in indicine cattle, but has been previously reported in low frequency in African taurine [17,18]. The identification of the rare variant $LALBA^{\mathrm{E}}$ in the Beninese cattle supports its recent description in the African zebu Butana as well as in some European and Asian breeds [9,52]. Tetens et al. [52] associated the presence of this variant in the Hinterwälder taurine breed with prior indicine introgression. The presence of this variant in some Somba cattle originated from zebu, considering the existing indicine introgression in the Somba cattle breed [45]. Nevertheless, the evaluation of the $LALBA^{E}$ distribution in other breeds is recommended. Additionally, we identified the missense SNP rs714688595C>T in Somba and crossbreed cattle. This polymorphism is known and annotated in the Ensembl variant table for the bovine *LALBA* gene. However, we found no study describing the p.Arg10Trp substitution in α-LA caused by this SNP. Indeed, α-LA$^{\mathrm{A}}$ diverges from α-LA$^{\mathrm{B}}$ by the AA replacement p.Arg10Gln. Further known AA exchanges in α-LA include the variants C (p.Gln>Glu, unknown position), D (p.Gln65His) and E (p.Ile41Val) [14,75,76], whereas we could not identify the causing SNP on DNA-level in our animal material. Therefore, we believe that SNP rs714688595C>T leading to p.Arg10Trp characterizes a new protein variant $LALBA^{F}$.

The detection of the SNP rs718675014C>T and rs110359174A>G in the 3′-UTR corroborates previous findings in the Butana cattle [9]. In addition, the functional effects of these polymorphisms in the bta-miR-143 and bta-miR-2467-3p, respectively, concur with the findings of Said Ahmed et al. [9]. However, we retrieved less miRNA binding sites or targets/seeds affected by the SNP. Several SNP were observed in the 5′-UTR of the *LALBA* gene, but they were not addressed in previous studies (Table S3). Many of these SNP are noteworthy as they affect TFBS or miRNA-binding sites, with potential influences on the differential expression of *LALBA* in the Beninese cattle.

4.1.6. LGB

The high frequency of LGB^{B} (corresponding to the reference sequence, see Table 1) supports previous findings in Beninese as well as in other African cattle breeds [17,18]. In contrast to Moazami-Goudarzi et al. [18] and Ceriotti et al. [19], we cannot confirm the presence of LGB^{A}. This variant is due to two missense SNP rs110066229G>A (p.Gly64Asp) and rs109625649C>T (p.Ala118Val), and the two synonymous SNP rs110180463C>T (p.Asn63) and rs110641366T>C (p.Asn88) [40,77]. As we could only identify the SNP rs109625649C>T and rs110641366T>C in our animal material, we propose that this is an intermediate *LGB* form, not described until now. Moazami-Goudarzi et al. [18] used isoelectric focusing to segregate β-LG$^{\mathrm{A}}$ from β-LG$^{\mathrm{B}}$, whereas it is not mentioned if the differences in the electrophoretic separation is caused by changes in the isoelectric point due to both amino acid exchanges p.Gly64Asp and p.Ala118Val, or only due to one of the amino acid exchanges. In the study by Ceriotti et al. [19], the typing of the protein variant LGB^{A} was based on the visualization of the SNP rs109625649C>T using the PCR-restriction fragment length polymorphism (RFLP) method. The second SNP rs110066229G>A was not analyzed using this method [78]. Therefore, it is possible that the animal material examined by Ceriotti et al. [19] did not contain the actual variant LGB^{A} but, as in our case, the allele LGB^{B1}. Ganai et al. [40] observed a complete linkage disequilibrium (LD) between the causal SNP of LGB^{A} and LGB^{B} in Dutch Holstein Friesian cows. Therefore, the omission of the SNP rs110066229G>A in the Beninese cattle breeds is unprecedented and may suggest the breakage of LD between the two major causal SNP of LGB^{A} and LGB^{B} in the Beninese cattle. Considering the existing information, we annotate the intermediate form of the *LGB* gene sequence caused by the missense SNP rs109625649C>T (p.Ala118Val) and the synonymous SNP rs110641366T>C (p.Asn88) as LGB^{B1}.

Another new protein variant detected in this study is the β-LG$^{\mathrm{K}}$. This variant is due to an insertion at BTA11:103257980 in exon 3 altering the reading frame, and inducing a premature stop codon (p.Thr92Asnfs*13). The mutated protein is shortened by 74 AA

(46%) and probably lacks domains that are essential for normal protein function. Although peptide deletions or insertions characterize some milk protein variants in ruminants, the loss of a large peptide sequence as observed in this study is uncommon [14,79,80]. Firstly, to our knowledge, no peptide skipping has been previously reported in the ruminant β-LG protein. Secondly, the largest peptide deletion identified in cattle associated the α_{s1}-CN^{A} with the loss of 13 AA caused by a single T>A SNP [81]. Further deletions of peptide sequence in cattle were associated with the α_{s1}-CN^{H} (peptide 51 to 58) in Kuri (an African crossbreed) and α_{s2}-CN^{D} (peptide 51 to 59) in European breeds due to exon skipping events [17,79]. In goats, the deletions of larger peptide sequences are more frequent with significant effects on milk protein genes. For instance, the caprine α_{s1}-CN^{F} (37 AA deleted) as well as several null alleles (α_{s1}-CN^{O1}, α_{s1}-CN^{O2}, α_{s2}-CN^{O}, β-CN^{O}) were associated with low or null expression of casein genes, respectively [79,82]. Therefore, we presume a high impact of the observed peptide skipping in the β-LG protein due to the described insertion in *LGB* exon 3. With regard to the indirect effect of the *LGB* gene on the total casein content [83,84], the variant *LGB*^{K} could affect animal milk composition and yield. The low frequency of the variant in the investigated population suggest a relative recent occurrence of the variant or indirect negative selection against animals carrying this variant. Further investigations should evaluate the functional impact of the *LGB*^{K} variant. Moreover, this protein variant is interesting given the high allergenic potential of β-LG, a milk protein missing in human milk [14]. For instance, camel milk containing no β-LG is recommended as an alternative milk protein source for cow milk protein allergy patients [85–87]. Similar observations were also made for the bovine *CSN1S1*^{G} and several null casein alleles in goats [83,88].

In addition to the missense and frameshift variants, we detected two previously not described synonymous SNP. Further investigations are imperative to clarify the impact of the new synonymous variants in the *LGB* gene. Indeed, Cartegni et al. [89] revealed potential effects of synonymous SNP on splicing. Various synonymous SNP are also linked to specific protein variants (e.g., *CSN2*^{A2}, *CSN2*^{A3}, *CSN3*$^{A'}$) [14].

The unique polymorphism observed in the 3′-UTR of *LGB* induces the loss of a bta-miR-7860 target site (Table 5). Regarding the polymorphisms in 5′-UTR of the *LGB* gene, two SNP promote the gain of TFBS, whereas the third alters a TFBS and the target sites of several miRNA. The SNP rs41255683G>C is in close proximity (at 34 bp) to SNP g.-215C>A (BTA5:103255964) causing a low expression level of *LGB* in cattle [90]. We were not able to detect the g.-215C>A SNP, but it seems to be rare, as it was similarly not found by Ganai et al. [40], and is also not referenced in the Ensembl variant table. Nevertheless, we mapped the SNP rs41255679C>G in the 5′-upstream region of *LGB*. This polymorphism was reported within the TF activator protein-2 (AP-2), and associated with differential expression of the *LGB*^{A} and *LGB*^{B} protein variants [40,41]. These findings evidence the necessity to demonstrate the potential effects of SNP positioned in 5′-upstream regions on milk protein genes.

4.2. Casein Haplotypes

The presence of the haplotype B-A1-A-B (corresponding to *CSN1S1-CSN2-CSN1S2-CSN3*) in about 50% of the Lagune and Somba cattle (Table 4), is in agreement with previous findings in the Beninese and African taurine breeds [17,18,42]. Bonfatti et al. [21] related haplotypes including *CSN2*^{A1} and *CSN3*^{B} with increased α_{s1}- and κ-CN milk contents, and reduced β- and α_{s2}-CN concentrations. *CSN2*^{A1} is negatively correlated with milk digestibility and human health [11,91]. In addition, haplotypes with the *CSN2*^{A1} were associated with low milk and protein contents [20,22]. Therefore, the low milk performance of the Beninese taurine cattle is possibly due to the high frequency of the haplotype B-A1-A-B [7]. Inversely, a high frequency of the haplotype B-A2-A-A was described for Holstein and several European taurine breeds [20,60], which favorably supports the importance of the *CSN2*^{A2} in bovine milk production. The latter haplotype was identified in low frequency in African taurine and the Sudanese Butana cattle [9,17,42], but not in the present study. Nevertheless, the distribution of the haplotype B-A2-A-B in moderate frequencies over all breeds, is interesting as it may be associated with appreciable milk performances and should be included in future Beninese cattle improvement strategies. This haplotype is similarly predominant in the Brown Swiss cattle population [20,60]. Moreover, we estimated a high frequency of the haplotypes B-A1-A-A and C-A2-A-B in the crossbreeds. Remarkably, the distribution of the latter haplotype in crossbreeds is linked to the high occurrence of $CSN1S1^{C}$ in indicine cattle. However, this haplotype differs with $CSN3^{B}$ from the most frequent haplotypes in indicine cattle (C-A2-A-A) [9,17]. These results suggest the presence of both indicine and taurine imprints in caseins regions in the crossbreeds. With regard to their association with milk performances, Boettcher et al. [20] related haplotypes comparable to B-A1-A-A and C-A2-A-B (i.e., excluding the *CSN1S2* gene) with higher milk protein and fat contents, and with lower milk yield in Italian Holstein and Brown Swiss Cattle. Considering that the $CSN1S2^{A}$ protein variant is the most prevalent in European taurine breeds, we assume that the haplotypes described by the latter authors correspond to B-A1-A-A and C-A2-A-B, and in consequence, we expect the same effects [13]. Finally, the casein haplotype B-A1-A-A was rare in African cattle, but dominant in British Friesian and German Black Pied Cattle [17,42,44,60].

5. Conclusions

The current study confirmed milk protein variants in Beninese cattle breeds as previously identified in commercial or outbred populations. As a novelty based on the analyses of sequence data, unknown polymorphisms including SNP and InDel, were detected. In this regard, the identified new milk protein variants are located in the *CSN3*, *LALBA* and *LGB* genes. Hence, the Beninese cattle carry favorable alleles as well as haplotypes, which can be used in genetic improvement programs for milk yield and milk quality of various breeds.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/genes12111702/s1, Table S1: Primer sequences utilized for the sequencing of the six milk protein genes (*CSN1S1*, *CSN2*, *CSN1S2*, *CSN3*, *LALBA*, *LGB*) in the Beninese indigenous cattle breeds; Table S2: List of the bovine miRNA and their corresponding seed sequences retrieved from the TargetScan website and used in "targetscan_60.pl" tool for the detection of miRNA target sites; Table S3: List of the polymorphisms detected within the exon sequences, flanking intron sequences and parts of the 5′-upstream regions of milk protein genes (*CSN1S1*, *CSN2*, *CSN1S2*, *CSN3*, *LALBA*, *LGB*) in Beninese indigenous cattle breeds; Table S4: Distributions of variant types (according to their positions and consequence) detected within each of the six milk protein genes (*CSN1S1*, *CSN2*, *CSN1S2*, *CSN3*, *LALBA*, *LGB*) and total in Beninese indigenous cattle breeds; Table S5: Functional consequence of the frameshift insertion of nucleotide A at BTA11:103257980 on the translational reading frame and on mature protein.

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Informed Consent Statement: Not applicable.

Data Availability Statement: All the DNA polymorphisms identified from our sequencing are submitted to the European Variation Archive (EVA) and are openly accessible at https://wwwdev.ebi.ac.uk/eva/?eva-study=PRJEB47999, accessed on 13 October 2021, [51]. Further data supporting the results of this article are presented in the Supplementary files.

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Publication 5. **Why and How European Farmers Are Dedicated to Breeding the Dwarf Dahomey Cattle**

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Why and How European Farmers Are Dedicated to Breeding the Dwarf Dahomey Cattle

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Simple Summary: The introduction of high productive cattle breeds in Africa is well known, but the contribution of African breeds to livestock biodiversity in Europe is generally overlooked. This study reports, for the first time, European farmers' interests in keeping the Dahomey cattle, and characterizes their management practices. The Dahomey cattle from Benin (West Africa) are the smallest cattle in the world, and they were introduced to Europe in the early 1900s. The findings revealed that European farmers are increasingly interested in keeping Dahomey cattle, because of their suitability for grassland maintenance and meat production, as well as their low management requirements (with regard to feeding, preventive and curative health care and reproduction management). Overall, the study displays the agricultural importance and ecological utilization of Dahomey cattle in European countries. It shows how small-sized cattle can support the promotion of sustainable livestock production and the management of ecosystems, including faunistic and floristic diversity.

Abstract: This study investigates the motivations and breeding practices of farmers keeping Dahomey cattle in European countries. Data were collected using a web-based open-closed questionnaire survey targeting 55 farmers from Germany, Switzerland and Austria. Descriptive analyses revealed that the earliest European Dahomey herds were established in 2005. Moreover, interest in the breed recently increased as 63.7% of the investigated farmers established their herds between 2016 and 2020. The average herd size comprises seven Dahomey cattle, kept for managing grassland (59.3%), for production of meat or as breeding stock (32.1%) and for a hobby (8.6%). The animals are mostly kept in grazing systems throughout the year, partly fattened with supplement feeds. The low disease incidence and no need for extra health care in the herds indicate the robustness of the breed. Furthermore, meat quality, calving ease, small size, calm character and low feed requirements of Dahomey cattle were valued by the farmers. For the preservation of these features, farmers confirmed their enthusiasm to support any breeding and conservation program of this smallholder breed in Europe and Benin. This study highlights the importance of small-sized cattle for sustainable breeding systems and with regard to ecosystem management practices.

Keywords: African shorthorn taurine; small-sized cattle; grassland values; breeding systems; smallholder; resistance to diseases; Benin

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1. Introduction

Dahomey is the former name of the Republic of Benin in West Africa. This name is also attributed to the smallest cattle worldwide, originating from Benin [1]. The Dwarf Dahomey cattle are characterized by an average wither height of 90 cm with an average adult body weight of 180 ± 30 kg for cows and 260 ± 40 kg for bulls (Figure 1) [1,2]. The small size of these shorthorn taurine (*Bos taurus*) animals is associated with easy calving, and with several favorable features including adaptation to marginal areas and resistance to diseases [1,3]. Dahomey cattle, known as Lagune cattle in Benin, are adapted to tropical environments through their original distribution to the rainforest and coastal regions of western and central Africa [4]. They are kept in Benin in challenging production systems for multiple purposes including socio-cultural functions (social status, sacrifices, bride wealth), draught (integration of livestock and crop production), means of saving or insurance and meat production, but not for milk production [4,5]. Unfortunately, the Lagune breed is increasingly threatened in Benin by indiscriminate crossbreeding with large-sized animals, and the lack of appropriate strategies or policies to promote and ensure its sustainable use [6]. In addition, the breed is poorly investigated and no information exists with regard to their population size in other African countries or in Europe [7]. Recent genomic characterization using medium-density SNP chips confirmed the Beninese indigenous Lagune breed as the closest ancestor of Dahomey cattle kept in Europe [8]. Similarly, applying phylogenetic analysis of the mitochondrial D-loop DNA sequence, Pfeiffer et al. [9] observed a large genetic distance between the Dahomey cattle and European taurine breeds.

Figure 1. A three years old Dahomey bull kept in Germany.

Dahomey cattle were introduced in diverse African (e.g., Gabon, Democratic Republic of Congo, Zambia) and European countries in the early twentieth century [4,9]. According to existing reports, the Antwerp zoo in Belgium was the first destination of Dahomey cattle in Europe [2]. To date, the breed is kept in several zoos and private herds in European countries [9]. The *Zootierliste* website [10] indicated 39 zoos in Europe keeping Dahomey cattle, including 23 zoos in Germany, five zoos in the Czech Republic and three zoos in Switzerland, two in The Netherlands, two in Austria, two in France, one in Belgium and one in Hungary. European farmers are organized in diverse breeding associations, including the *Verband Europäisches Dahomey-Zwergrind* (VEDZ; European Association for Dahomey Dwarf Cattle) [2]. This association, founded in 2001, currently includes 77 breeders from Germany, Austria, Switzerland and the Czech Republic [2]. In addition, further national associations of Dahomey cattle breeders include the *Verein Dahomey Schweiz* in Switzerland and the *Dahomey Zuchtverband Deutschland e.V.* in Germany [11]. The later association, the most recent to our knowledge, was founded in July 2020 [11].

The diversity of Dahomey cattle herds and breeding associations in Europe reflect an increasing interest in the breed. The VEDZ reports an ongoing application procedure for the registration of the breed in the German catalogue for cattle breeds. Young animals or reproducers of Dahomey cattle are regularly marketed, also on online platforms. Yet, the breed is barely addressed in scientific studies. Golze [12] recently compared meat quality traits of

11 cattle breeds in Germany and reported higher organoleptic characteristics (including tenderness and special flavor) for Dahomey cattle. In addition, the meat of Dahomey has valuable nutritional content, because of the high proportion of absorbable iron and the low cholesterol content. Similar valuations for Dahomey cattle meat are reported on the website of several breeders and breeding associations [11,13–15]. Golze [12] related the appreciable nutritional characteristic of Dahomey cattle meat with the low growth rate of the animals and the feeding system characteristics. Moreover, the adaptation of small-sized cattle to marginal or harsh production systems is well acknowledged [1]. In the ongoing global context of climate change, the Dahomey cattle may represent a good alternative for sustainable cattle farming under limited resources [1]. Conventional livestock production systems are increasingly reported worldwide for their negative impacts on the environment, including the degradation of grasslands (i.e., vegetation communities dominated by herbaceous) [16]. In Europe, grasslands are largely reliant on regular removal of aboveground biomass by agricultural land use, including grazing and/or mowing [17]. Grassland maintenance practices aiming at conserving floristic biodiversity and enhancing the sustainability of land ecosystems are therefore required. European agricultural policy and researchers recommend low pressure on grasslands with seasonal pastures, and the use of specific animal species or breeds presenting specific grazing behavior or low feeding requirements [16,18,19]. Hence, the Dahomey breed should be promoted, stimulating scientific investigations and political support in this regard. The current study characterizes for the first time the breeding systems of the Dwarf Dahomey cattle in Europe. Farmer interests, their satisfactions and suggestions for the promotion of this breed are subsequently addressed.

2. Materials and Methods

2.1. Data Collection

An open-closed questionnaire focused on socio-economic characteristics, the interest of farmers in keeping Dahomey cattle, the establishment of Dahomey herds and the membership in breeding associations, acquisition of initial herds (origin, price of the animals), initial and current herd sizes and herd composition, management system characteristics and management constraints, animal exploitation and performances, as well as farmer suggestions for the promotion of Dahomey cattle. Socio-economic characteristics included the farm location, age of farmers and their professional activity and experience in cattle breeding. Herd composition comprised the respective number of calves, bulls and cows, and the presence of other breeds in the herd. The herd management system was surveyed with regard to housing, feeding, reproduction and health management practices. The performance evaluation of animals included body weight and the female fertility traits age at first calving and calving interval. As Dahomey cattle are not used for milk production, no records were available for milk or protein yield. However, farmers were asked to rate milk production, animal growth, meat quality and fertility of Dahomey cattle using the grades "high", "reasonable", "low", "very low". In addition, farmers categorized their appreciation (i.e., "like", "do not like" or "no appreciation") for specific characteristics of Dahomey cattle including the calm character, calving ease, feeding requirements, disease resistance and the small body size. Finally, the farmers indicated their overall satisfaction with the Dahomey breed with a grade between 5 and 1 (5 = highly satisfied, 1 = not satisfied).

The questionnaire (see data availability section) was prepared on the "Google Form" online platform and submitted to the members of the VEDZ (77 members in total) [2] and the "*Verein Dahomey Schweiz*" (50 members in total) between Mai and October 2021. Further known Dahomey breeders without a breeding organization membership, were invited. A total of 55 farmers voluntarily participated in the survey. They represent 43% of the members of the surveyed breeding associations.

2.2. Data Analysis

The data were analyzed with basic R packages including *dplyr* and *tidyr* [20,21], and using elementary descriptive statistics, i.e., frequency distributions for qualitative variables and means for quantitative variables. All the graphs were prepared with the *ggplot2*, *viridis*, and *viridisLite* packages [22–24].

3. Results

3.1. Socio-Economic Characteristics and Motivations of the Farmers

The majority of the respondents resided in Germany (60.0%), followed by Switzerland (36.4%) and Austria (3.6%). They were mainly men (72.7%) and were in majority between 40 and 60 years old (Figure 2A). Most of Dahomey holders (64.2%) were not professional farmers, and a fraction of 94.3% was professionally active in an off-farm occupation. Farmers' experience in cattle breeding varied between one and 16 years. Two farmers breed Dahomey cattle since 2005. A total of 63.6% of the respondents adopted the breed between 2016 and 2020 (Figure 2B). In addition, 85.5% of the respondents are members of a breeding association, and 69.6% of them received their membership during the past five years. Motivations for holding Dahomey cattle included grassland maintenance (59.3%), reproduction and meat production (32.1%) and hobby farming (8.6%).

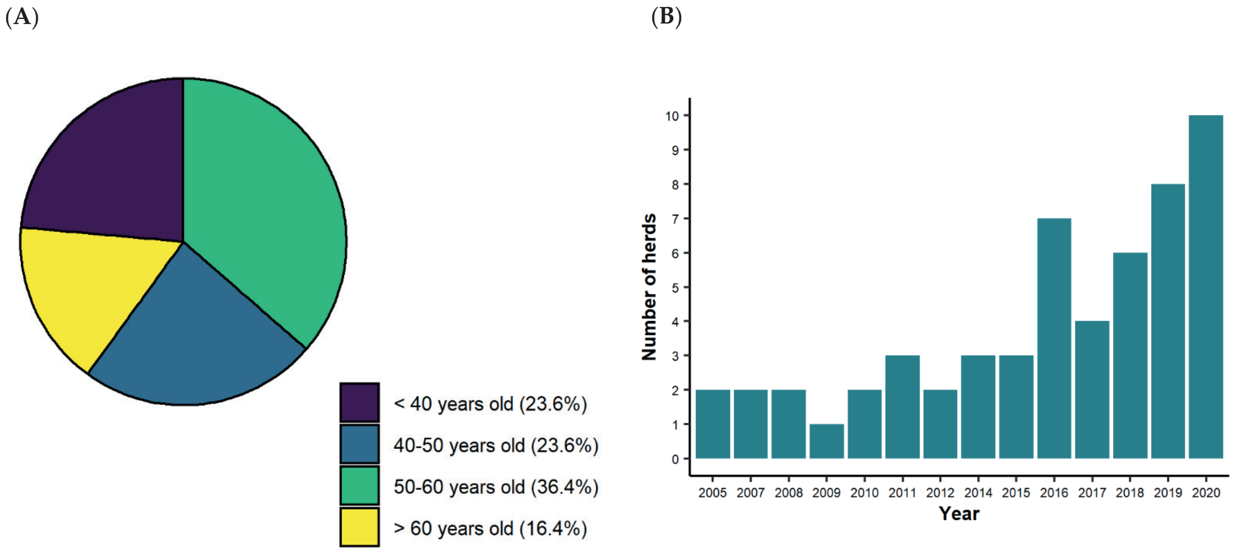

Figure 2. (**A**) Distribution of the investigated European Dahomey farmers according to age groups; (**B**) Number of established Dahomey cattle herds per year between 2005 to 2020 in Germany, Austria and Switzerland, indicating the development of European farmers' interest in the Dahomey cattle breed.

3.2. The Dahomey Cattle Herds

3.2.1. Acquisition of Initial Stocks: Origins and Prices

Dahomey farmers established their initial breeding stock with one to 10 animals (three animals on average). The animals were mainly acquired from fellow farmers (69.1%), through the VEDZ (21.8%) or on online platforms (5.5%). Only two farmers (3.6% of the respondents) acquired their initial Dahomey cattle from zoos, namely the *Tierpark Brüggen* and *Zoo der Minis Aue e.V.* Figure 3 displays the distribution of the acquisition price. The majority of the animals were purchased for 500 to 1000 Euros. Cows represented the most expensive category, costing more than 1000 Euros.

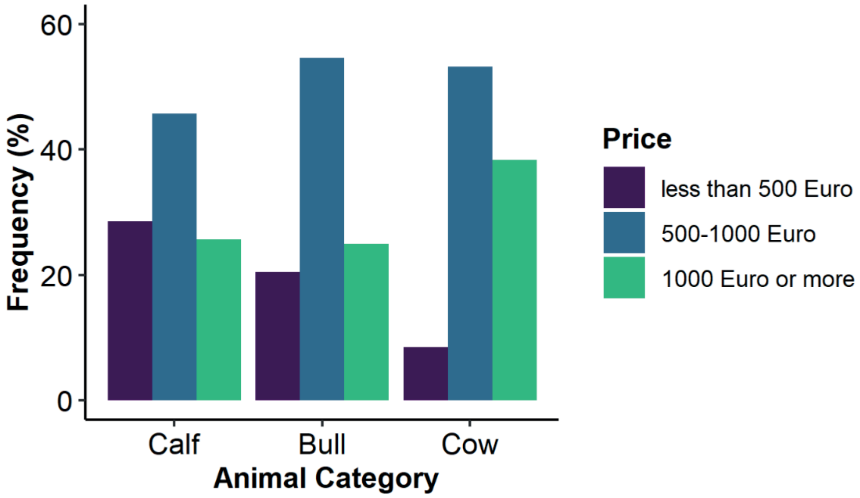

Figure 3. Acquisition price of Dahomey cattle for calves, bulls and cows in Germany, Austria and Switzerland. Calf = cattle less than one year old; bull and cow = reproductive male and female cattle, respectively.

3.2.2. Herd Sizes and Composition

Dahomey cattle herds consisted of two to 23 Dahomey animals, with an average of seven animals per herd. Cows and bulls were the predominant categories. Calves and young cattle were absent in more than 20.0% of the herds (Figure 4). A total of 83.6% of the investigated herds only consisted of Dahomey cattle. The remaining herds (16.4%) included additional breeds such as Angus, Belgian Blue, Limousin and crossbreeds with Dahomey.

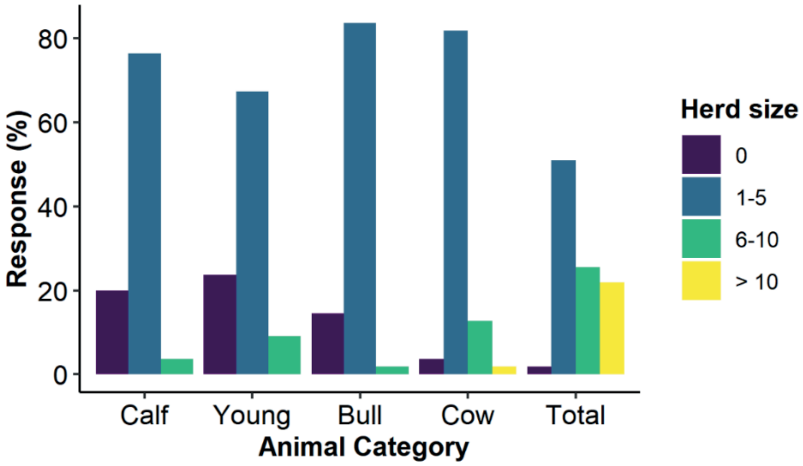

Figure 4. Herd size and structure of the investigated Dahomey cattle in Germany, Austria and Switzerland. Calf = cattle less than one year old, young = cattle between one and three years old, bull and cow = reproductive male and female cattle, respectively.

3.3. Herds Management

3.3.1. Housing and Feeding

The housing and feeding systems of Dahomey cattle varied according to seasons (Table 1). Animals were mainly kept on pasture or in open barns. The open barn system with protections against cold floor, snow and rain using windbreak were the predominant housing mode in winter. In 58.2% of the investigated herds, access to pasture was combined with open or closed barns during all seasons. A fraction of 34.6% of the respondents kept Dahomey cattle on pasture during the whole year, apart from the winter months.

Table 1. Housing and feeding management systems in different seasons in the Dahomey cattle herds from Germany, Austria and Switzerland.

	Season			
	Winter	**Spring**	**Summer**	**Autumn**
Type of housing (%)				
Open barn	53.6	36.0	31.1	34.7
Only on pasture	26.1	54.7	62.2	58.3
Closed barn	20.3	9.3	6.8	6.9
Type of feeding (%)				
Pasture	18.7	46.0	58.4	50.5
Own produced feed	52.8	36.0	25.8	31.6
Purchased feed	14.3	9.0	5.6	8.4
Supplement	14.3	9.0	10.1	9.5

In addition to the utilization of natural grazing systems, animal feeding in Dahomey herds included own produced or purchased feed and supplements (Table 1). Own produced feed was the most prevalent feeding component in winter. Feeding strategies in Dahomey cattle focused on grassland maintenance through free and rotational grazing (74.6%), but also to improve meat production (25.5%).

3.3.2. Reproduction and Health Management

Natural mating was very common in Dahomey cattle herds. A small fraction of 5.5% of farmers used artificial insemination accomplished by veterinarians or technicians. Nevertheless, three-fourths of the respondents selected herdbook bulls (76.4%) and registered their animals in herdbooks (74.6%). Castration of bulls was applied in 45.5% of the herds during the first or second age year.

A quite large fraction of 32.7% of the respondents indicated the absence of frequent animal diseases. Nevertheless, diarrhea was the most reported disease symptom (in 40.0% of the investigated herds). Further rare diseases included pneumonia, coccidiosis and parasitic infestations. Almost all Dahomey farmers (92.7%) controlled the health status of their animals once per day. Curative treatments were generally rare in Dahomey cattle (Figure 5). Therapies against parasitic infestations were the most frequent in the herds (32.7% of the respondents), while hoof care was very rare or has never been applied in 94.6% of the investigated herds. Similarly, 72.7% of the Dahomey farmers have never vaccinated their animals. Moreover, a particular or sudden case of animal death has never been experienced in 92.7% of the investigated herds.

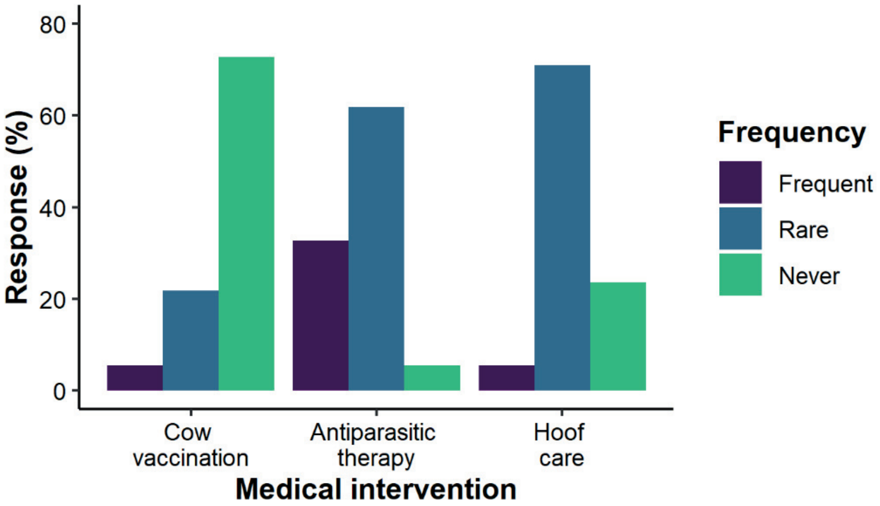

Figure 5. Frequency of medical interventions in Dahomey cattle herds from Germany, Austria and Switzerland.

3.3.3. Animal Exploitation

Dahomey cattle were slaughtered frequently and occasionally in 7.3% and 45.5% of the surveyed herds, respectively. Forty percent of the surveyed farmers reported an average of one to four animals slaughtered per year. Meat production (5.5% of the respondents) and herd management factors (40.0% of the respondents) were the main reasons for animal slaughter. The later factors included reduction of the herd size, impossibility to sell the animal, infertility or availability of another bull, animal character (e.g., aggressive), age and health status. Dahomey animals were generally slaughtered between their second and third year of life (29.1% of the respondents).

The majority of investigated farmers reported that they sold Dahomey reproducers frequently (30.9%) or occasionally (41.8%). Animal prices varied according to sex, and Dahomey cows generally valued more than 1000 Euros (51.2% of the respondents). In contrast, only 30.2% of the respondents described such a price range for bulls. According to 51.2% of the farmers, the price for Dahomey bulls ranged between 500 and 1000 Euro.

3.3.4. Animal Performances and Farmers' Valuations

Meat quality and cow fertility in Dahomey cattle were highly rated by 40.0% and 23.6% of the Dahomey farmers, respectively (see Figure 6). In contrast, 58.2% of the surveyed farmers reported low or very low milk performance in the breed. In contrast, 56.4% of the respondents rated Dahomey cattle growth (body weight gain) as reasonable. The average adult weight of Dahomey cows (> two years old) ranged between 200 and 300 kg (65.5% of the respondents), while adult bulls weighed 300 kg on average (according to 36.4% of the farmers).

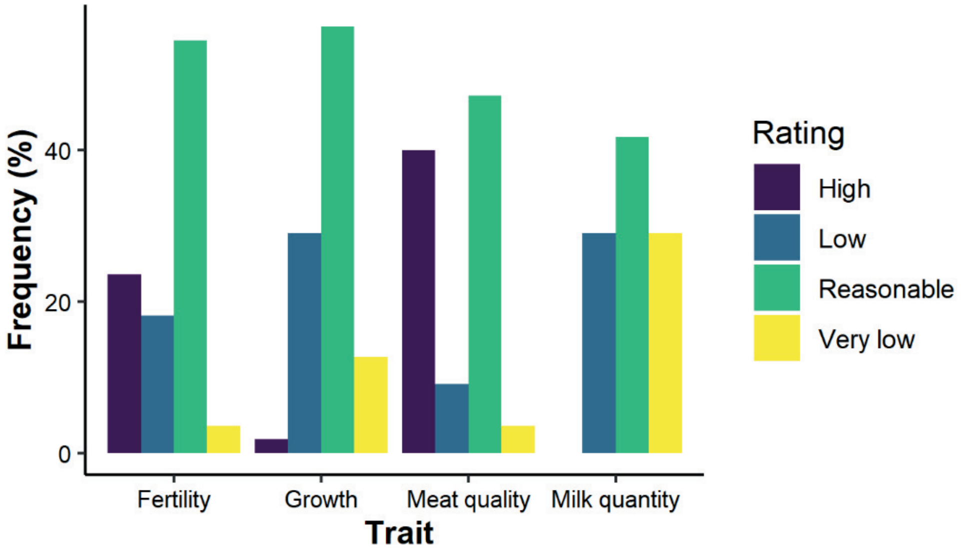

Figure 6. The ratings of European farmers for the Dahomey cattle performances.

In 80.0% of the investigated herds, the age at first calving of cows was between 24 to 36 months. Nevertheless, some farmers (18.2%) reported an age at first calving below 24 months. In most cases (70.9% of the respondents), the average calving interval was about one year. Calving was generally year-round in the majority of the herds (52.7%), but was more frequent in spring and autumn (36.4%) or summer (9.1%) in some herds.

Overall, 60.0% and 32.7% of the respondents rated the performance of Dahomey cattle with the satisfaction grade of 5 and 4, respectively. Traits mainly appreciated in the Dahomey cattle included the calving ease (100% of the respondents), resistance to diseases (98.2% of the respondents), small size (94.6% of the respondents), calm character (83.6% of the respondents) and low feed requirements (70.9% of the respondents).

3.4. Promotion of the Dahomey Cattle and Breeding Constraints

Farmers expressed their willingness to support eventual breeding programs in Dahomey cattle in Europe (85.5%) and in Benin (72.7%). They recommended selection strategies in Dahomey cattle focusing on meat quality and quantity (81.8% and 49.1% of the respondents, respectively). However, 29.1% of the farmers emphasized the necessity to preserve the originality of the breeds including the small body size, female fertility, robustness, resistance to diseases and coat color. Moreover, the farmers suggested the promotion of the Dahomey cattle mainly through advertisement (52.7% of the respondents) and the reinforcement of breeding associations and partnerships (7.3% of the respondents).

Most of the surveyed farmers (61.8%) reported no constraints with regard to breeding aspects of Dahomey cattle. The few constraints identified by some farmers included the shortage of grazing land, the high slaughter costs, the aggressiveness of some bulls, the lack of insemination facilities, and the limited support from agricultural authorities. In addition, some farmers complained about the consideration of Dahomey cattle as one livestock unit (LU) like larger cattle breeds.

4. Discussion

4.1. The Interests of European Cattle Breeders in the Dahomey cattle

The current study identified an appreciable number of farmers interested in keeping Dahomey cattle, although they represent a minor fraction of the overall beef farmers in Europe (e.g., 49,675 beef farmers in 2021 in Germany) [25]. A large number of Dahomey farmers from Germany may be related to the creation of the breeding association (VEDZ) 20 years ago in the country [2]. Moreover, the presence of Dahomey cattle in 23 zoos in Germany enhanced the breed attractiveness [10]. The oldest Dahomey cattle herd reported in this study was established in 2005. However, due to the establishment of the VEDZ in 2001 [2], earlier Dahomey cattle herds may exist. Additionally, Dahomey breeders from Austria and Switzerland are reported in this study. The young breeding association (*Verein Dahomey Schweiz*), established in Switzerland two years ago, reflects the numerous farmers investigated in this country. Further Dahomey cattle farmers are located in other European countries including the Czech Republic [2], but they did not participate in this survey. The increasing number of farmers adopting Dahomey cattle, as observed in the past five years, indicates a further expansion of the Dahomey cattle population in the next years.

The findings revealed that Dahomey cattle are mainly kept for grassland maintenance. The suitability of the breed for grassland maintenance is due to the small body size and low feed requirement [1]. The Glanrind, Rotes Höhenvieh and Limpurger breeds are similarly reported for grassland maintenance in Germany [26,27]. However, in contrast to Dahomey, these breeds are characterized by decreasing population size in Germany [26,27]. Cattle husbandry in extensive grazing systems is described as a sustainable nutritional strategy to reduce the negative impacts of livestock production on the environment [28]. Researchers described an increasing enthusiasm for extensive cattle grazing systems in European countries [29,30]. For instance, Bunzel-Drüke [31] reported an “all-year grazing project” with Heck cattle for the sustainable management and the conservation of the natural grassland in Central Europe. According to Schley and Leytem [29], cattle breeding for grassland maintenance offers outstanding convenience for the sustainable management of ecological niches and the promotion of faunistic and floristic diversity. Likewise, Tóth et al. [30] observed that cattle grazing induces a more species-rich and trait-rich vegetation with the higher cover of forbs, in comparison to sheep grazing. Sheep have a highly selective grazing behavior, and they are more sensitive to environmental conditions affecting pasture quality and grazing time than cattle [32]. The availability of small-sized Dahomey cattle that are suitable for grassland maintenance is therefore a strong advantage in this regard. In addition, the Dahomey cattle breed fulfills further breeding objectives including meat production. Cattle grazing systems will be promoted in European countries such as Germany, because of the higher consumption of cattle meat compared to sheep meat [33]. One-third of the investigated farmers confirmed that they use Dahomey cattle for meat production. Similarly, multi-purpose use is known for the Rotes Höhenvieh cattle breed [27]. Rotes Höhenvieh cattle are of medium size and able to produce under harsh environments, similarly to Dahomey cattle [34].

The interest in breeding medium or small sized cattle is not uncommon. Several small sized cattle breeds attracted farmers and scientists worldwide [35]. For instance, the Dexter, small cattle from Ireland, are kept and preserved as purebred. Other cattle breeds including the miniature Hereford, Lowlines (small Angus cattle), Jersey or Zebus are exclusively selected for their small size [35]. According to Boden [35], reasons to keep small sized cattle include their easier management requirements, i.e., regarding feeding (pasture), housing, labor time, etc. [35]. This observation is in line with the dominance of non-professional farmers (with off-farm occupation) in the sampling design from the present study. In addition, more than 50.0% of the surveyed farmers were more than 50 years old, which concurred with the appropriateness of small sized cattle for elderly people seeking to invest in cattle [35]. Because the Dahomey cattle require low management resources, they represent a convenient option for future cattle farming systems [1]. Indeed, the increasing scarcity of agricultural resources (e.g., land) in several areas is well acknowledged [35]. Accordingly, small sized cattle are promoted for local urban farming or challenging environments (e.g., extreme temperatures) [35]. These observations may stimulate researchers and policy makers, especially in Benin, to preserve and promote indigenous cattle breeds despite their small body size.

4.2. Breeding Systems for Dahomey Cattle in European Herds

The management systems of Dahomey cattle in the investigated herds are in accordance with their main production objectives (i.e., grassland maintenance, meat production), and their robustness. Dahomey cattle are mainly kept in small herds (seven animals on average) under extensive grazing systems all year round, and with almost no health preventions and treatments. In addition, reproduction management mainly involves natural mating with less intervention from veterinarians. The management system of Dahomey cattle is comparable with the one of the Lagune (the ancestor of the Dahomey cattle) in Benin. Similar management systems were observed in European cattle herds targeting beef production, grassland maintenance, hobby farming or organic cattle breeding [29]. Accordingly, a surveyed farmer from Germany favored Dahomey cattle breeding for organic production systems. Consequently, the Dahomey breed is a valuable alternative for organic farming, which is continuously increasing in Europe.

Animal slaughtering and sale of breeding stock indicate the economic importance when keeping Dahomey cattle. The associated costs are quite small, due to the low management requirements. The rarity of diseases as well as the lack of proper preventive and curative health care corroborate the disease resistance of the breed [3]. The exchange of Dahomey cattle, mainly among fellow farmers, and the small population size of the European Dahomey cattle, are reasons for the high genomic inbreeding [8]. The inclusion of reproducers from zoos may reduce inbreeding in the population and increase the effective population size. Preliminary evaluation of the Dahomey cattle population kept in zoos is imperative in this regard. Moreover, the willingness of several farmers to register their animals in herdbooks is a relevant opportunity to manage genetic diversity in the Dahomey population [8]. Pedigree information from herdbooks is also beneficial for establishing breeding strategies and preserving the breed.

4.3. Promotion of Dahomey and Lagune Cattle Breeds

The reported performances of the Dahomey cattle are in line with the breed valuations by the farmers. The age at first calving between two and three years, as well as the calving interval of one year, illustrate the high fertility status of the Dahomey cows. Similar reproductive performances are reported for Lagune cattle in Benin [5]. Likewise, for several cattle breeds kept in Germany [36,37], the age at first calving and calving interval varied between 24 to 36 months, and 361 to 380 days, respectively. Moreover, the average adult weight of about 300 kg concurs with the reasonable growth and low feed requirement of this breed, as known for Lagune cattle [38]. Despite their low average body weight, farmers reported that Dahomey cattle are categorized using the standard LU system (like large cattle breeds). Indeed, German agricultural policies attribute LU coefficients to animals based on their species, age and sex, whereby an adult cow and bull (over two years old) is equivalent to 1.2 LU, a heifer (female young cattle between one and two years old) represents 0.6 LU, a bull-calf (between one and two years old) represents 0.7 LU and a calf (female or male up to one year) represents 0.3 LU [39]. Yet, an adult Dahomey cattle (with an average body weight of 300 kg), would correspond to 0.6 LU following the scientific definition of one LU, which is equivalent to 500 kg body weight [40,41]. Against this background, the revision of classification systems for LU will support the promotion of small size cattle breeds like Dahomey.

In addition to the easy feeding management of Dahomey cattle, most of the surveyed farmers valued the meat quality. Comparable valuation for meat quality is reported for Beninese local breeds and the Rotes Höhenvieh, which are kept in similar grazing systems in Germany [42,43]. The phenotypic performances in a favorable range of Dahomey cattle justify the overall satisfaction of the farmers and their enthusiasm to promote this breed. In this regard, the current organization of the farmers in breeding associations definitely will facilitate breeding program improvements. Lagune breeders in Benin may benefit from the rich experience of the Dahomey breeding associations to Lagune cattle. For instance, the creation of collaborations between the VEDZ and Beninese farmers will contribute to knowledge exchange. Such programs should be initiated because the majority of the investigated Dahomey farmers confirm their enthusiasm with regard to the promotion of the Dahomey breeds in Benin and Europe. The current study constitutes a good starting point for such purpose, as it increases the visibility of the breed as suggested by the farmers.

5. Conclusions

Dahomey cattle have a great potential to be promoted in European countries because of their suitability for grassland maintenance. The association of cattle breeding with ecosystem management is of increasing importance, especially due to the ongoing global environmental challenges. Moreover, the easy management of the Dahomey cattle, associated with their disease resistances, their fertility and their meat quality are very valuable for cattle farming in small-sized herds, including meat production. Despite their importation to Europe, the breeding systems and performances of Dahomey cattle are similar to those of their ancestors, i.e., the Lagune, in Benin.

Author Contributions: Conceptualization, methodology, data curation and analysis, S.F.U.V.; Review and validation of the questionnaire: K.H., K.B. and L.H.D.; writing—original draft preparation, S.F.U.V., S.O.H.; writing—review and editing, S.O.H., I.J.G., K.B., L.H.D. and S.K.; supervision, L.H.D. and S.K.; funding acquisition S.F.U.V. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The survey was completely anonymous with no personal information being collected. The participation to the survey was fully voluntary and participant answers were confidentially processed. Therefore, no ethical approval were required.

Informed Consent Statement: All individual participants were sufficiently informed about the aims and the framework of the survey. They have voluntarily agreed to take part in the online survey and have given their consent for publication of the results.

Data Availability Statement: The raw data supporting the results of this article are stored at the server of the University of Giessen and will be made available upon reasonable request in accordance with data protection regulations.

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Conflicts of Interest: The authors declare no conflict of interest.

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3. General Discussion

3.1. Adaptive and Productive Features in the West African Indigenous Cattle Genetic Resources

This study displays a large variety of valuable features in the West African indigenous cattle breeds in Benin including the Lagune/Dahomey, Somba, and their crossbreeds (Borgou and Pabli); and demonstrates the need to ensure their improvement and sustainable management. More specifically, adaptive and productive traits have been characterized in the breeds, in line with previous knowledge on African animal genetic resources (AnGR).

3.1.1. Adaptive Features in West African Indigenous Cattle breeds

Adaptability of an animal is the ability to survive and reproduce within a wide range of environments or under variable stressors [1–3]. In this regard, a large variety of agro-ecological zones and production systems characterizes the environment of the Beninese indigenous cattle breeds [4,5]. Further, researchers reported dynamic ecological, sociological, economic and political changes in the Beninese pastoral areas [5–7]. Animal adaptability may be illustrated with adaptation to humans, to extreme (hot/cold) climatic conditions, to scarcity of feed, water and further production resources; and tolerance or resistance to diseases [8]. The adaptation of the Beninese cattle to humans is evidenced by the high socio-cultural relationship between the Beninese farmers and their cattle (see Publication 1) [9]. Another example is the calm temperament of the Dahomey cattle breed, which is highly appreciated by the European farmers (Publication 5). The keeping of the Dahomey cattle outside (on pasture or open stable) under European climatic conditions (as reported in Publication 5), demonstrates the ability of the animal to cope with varied climatic conditions, including fluctuating temperature regimes across the temperate seasons. Indeed, the temperate climate in Europe contrasts with the tropical climate, characterized by high heat stress (with high temperature and humidity), in which the local cattle breeds are regularly kept in Benin. Yet, Dahomey cattle show good adaptation to their new environment. In terms of livestock production, European countries are experiencing environmental degradation, and cattle grazing systems are increasingly valued for ecological purposes [10]. The results of this study show the suitability of the Dahomey breed for such an extensive breeding system. Similarly, the low input systems in which cattle breeds are managed in Benin concur with their ability to survive and reproduce under limited resources. Beninese cattle breeding environments are characterized by important feed and water scarcity [11,12]. Animal feed is mainly made of natural pasture and farmers rarely provide feed supplementation [5,13]. The breeding of the Beninese cattle breeds in extensive systems is also associated with low management time and less sophisticated housing. Such systems are suitable for poor farmers, whose livelihood and subsistence depend not only on livestock husbandry but also on crop farming [5,13,14]. Finally, the resistance or tolerance to diseases (e.g. trypanosomiasis) in the Beninese taurine (Lagune/Dahomey and Somba) as reported in previous studies [15,16] are approved by current findings. Animal resistance or tolerance to diseases are important, as they imply low use of medical drugs, and consequently low production costs, less drug resistance and better quality of animal products (meat and milk) [17,18]. Higher resistance to diseases is observed in the Beninese shorthorn taurine in comparison to the longhorn taurine N'Dama, which has been more investigated in West Africa for their adaptive features [15]. Researchers relate adaptive traits in the Beninese shorthorn taurine to their evolution in the rainforest regions of central and western Africa [16]. Furthermore, adaptive features in the breeds are associated with farmers' breeding and selection practices [19]. In this regard, the current investigations in Benin and in Europe confirm previous findings that farmers highly value adaptive traits in West African cattle breeds [20,21].

3.1.2. Productive Features in West African Indigenous Cattle breeds

Productive traits in cattle breeding are generally related to meat and milk production. The taurine cattle (Somba and Lagune) in Benin produce less milk and are smaller-sized than the crossbreeds, especially the Borgou. Several other West African breeds such as the Zebu White Fulani, Gudali or Azawak present more milk and meat than all the Beninese indigenous cattle breeds [22–24]. However, the lower growth rate and milk yield in shorthorn taurine breeds may be associated with their low requirements in production resources, especially in feed and water. Most productive breeds such as the Gudali or Azawak are known for their higher requirements in feed and water [19]. A formal comparative study is therefore required to evaluate the production efficiency of the Beninese cattle and clarify the perception of their low productivity. Moreover, the quality of meat and milk in the shorthorn taurine are highly appreciated in Benin and Europe, in line with their low growth rate [13]. Accordingly, Kinkpé et al. [25] reported higher economical values for the shorthorn taurine in Benin in comparison to Zebu breeds (e.g. Gudali, Fulani breed, Red Fulani). Therefore, formal study is also expected to compare the organoleptic characteristics of meat and milk in the Beninese taurine, crossbreeds and further Beninese cattle breeds. Finally, Beninese (or in general, West African) cattle breeds should not only be evaluated based on their productivity in meat and milk, because they are bred and selected by smallholders for multiple purposes, including socio-cultural use (coat color, horn shape, etc.) [9,20,26]. The ecological benefit of these breeds, as shown by the Dahomey cattle in European countries, is also valuable and should not be overlooked. Several multipurpose European breeds are also characterized by lower performance in comparison to the breeds which have been regularly selected for milk or meat [27].

The current study indicates the need to promote the Beninese indigenous cattle breeds with respect to their actual agricultural, sociological and ecological worth. The importance of African AnGR for food security and the livelihoods of many farmers is frequently described [21]. Simultaneously, there is a need for increasing meat and milk production to meet the fast growing demand in foods of animal sources [28]. Nevertheless, Hyman et al. [29] observed that African farmers discount durable benefits of natural resources at a high rate in contrast with conventional economic theory. By doing so, African farmers favor the long-term use of natural resources [29]. This aspect should be considered for the sustainable use of the West African cattle breeds. For this purpose, adaptive features should be conciliated with productivity, as increasingly observed in developed countries, with regard to the global climate challenge [30,31].

3.2. Genetic Architectures of Adaptive and Productive features in the West African Indigenous Cattle Breeds

The current study identified several genetic patterns underlying adaptive and productive features, using different methods including selection signatures (Publication 2), genome wide association study (GWAS, Publication 3) and next generation sequencing (Publication 4).

3.2.1. Genomic Regions Affected by Selection Pressures

Selection signatures scan is common to investigate genomic footprints, left by adaptive processes to the production environment as well as by farmer selection practices [32]. The analysis is the most applied method to genetically characterize African cattle breeds [33–37]. The current findings agree with previous reports in other West African cattle breeds including the N'dama and the Muturu [35–38]. Several selective sweeps identified in Publication 2, and their associations with heat tolerance, immune response (tick resistance, trypanotolerance) corroborate known selective signatures due to the production environment of the indigenous cattle breeds in Benin [36–38]. In addition, specific regions under selection (e.g. BoLA class IIb genes such as *DSB*, *BOLA-DYA*, which were not previously reported in other West African cattle breeds) were observed and are associated with immune responses. These findings suggest further genetic investigation on the specific adaptive features of the Beninese cattle breeds. Moreover, the association of selective sweeps in Beninese cattle with quantitative trait loci (QTL) related to productive features (e.g. body length or weight, carcass traits), illustrates farmers' orientation towards increased meat and milk production [26]. As evidence, the latter trend is more observed in crossbreeds, described as more productive than the taurine (see Publication 1). Similar results are also observed in other African cattle crossbreeds including East African Shorthorn hybrids [39,40]. The crossbreeds in Benin display more selection signatures involved in heat stress adaptation than the taurine. These findings are in line with those reported in previous genetic studies addressing (West) African Zebu and crossbreed cattle, and confirm thermoregulation as an important adaptive feature of Zebu cattle [37,41,42].

3.2.2. Diversity and Functionality of Genomic Regions Associated with Morphometric Traits

Unlike selection signature analysis, GWAS is rarely reported in African cattle breeds, because of the difficulty to collect valid phenotypic data in extensive livestock production systems. Based on the existing information, the current study is the first using multi-breed GWAS to associate animal performance traits with genetic polymorphisms in West African cattle. Nevertheless, Yougbaré et al. [43] recently reported a similar GWAS study in purebred and crossbred Baoulé from Burkina Faso, and identified several genomic regions associated with trypanosome prevalence and morphometric traits.

The multi-breed GWAS for six morphometric traits (height at withers, sacrum height, heart girth, hip width, body length, and ear length) in the Beninese indigenous cattle breeds identified different associated loci. Several of the detected regions are well known for association with body traits (e.g. *VEPH1, PIK3R1 PTAFR*) but others are new (e.g. *CNTNAP5, EYA3*). The functional analysis of the detected regions revealed that they are not only related to carcass and growth traits, but also to adaptive features including feed efficiency, stress or immune response. These results are in line with the findings describing regions under selection pressures in Publication 2, and they substantiate the strong correlation between adaptive and productive traits in the Beninese indigenous cattle breeds. Our findings corroborate the observations of Kabi et al. [44] that African farmers select their cattle for adaptation to different agro-ecological fitness based on animal morphometric traits. This overlap of genomic regions associated with adaptive and morphometric traits is similarly reported in other studies [45–47], and justifies the assessment of adaptability in tropical cattle by measurable traits such as growth [1].

3.2.3. Genomic Variability in Milk Protein Genes

Another novel method applied in this study is the next-generation sequencing targeting milk protein genes of Beninese cattle breeds. The findings in Publication 4 detected several genetic polymorphisms (SNP, InDel and haplotypes) in the milk protein genes as previously reported in other West and central African breeds [48,49]. The distribution of some milk protein variants in the investigated breeds suggest possible association with milk traits. For instance, milk protein variants (e.g. β-CN^{A1} and κ-CN^{B}) or haplotypes commonly related to technological quality of milk were more predominant in the taurine than in the crossbreeds, whereas milk protein variants (e.g. β-CN^{A2}) associated with milk yield were more present in the crossbreeds [50–52]. The collection of phenotype data related to milk traits (e.g., milk yield and/or composition) in the Beninese cattle breeds will help to confirm these findings. Moreover, some specific milk proteins (α_{s1}-CN^{C}) known for beneficial effects on human health should also be promoted in the Beninese cattle breeds [53]. Furthermore, the detection of several previously unknown milk protein polymorphisms (κ-CN^{K}, α-LA^{F}, β-LG^{B1}, β-LG^{K}) support the unicity and genetic diversity of the West African cattle breeds.

3.3. Implications for the Promotion, Sustainable Use and Improvement of West African Indigenous Cattle Genetic Resources

Sufficient genomic information on AnGR are required to identify and to define adequate and rapid strategies for the improvement of the African livestock sector, and researchers are increasingly recommending the application of novel genomic tools to investigate African AnGR [28,54–56]. The current study contributes to filling the gap of knowledge in West African indigenous cattle genetic resources, and offers new perspectives for their sustainable use and genetic improvement [32]. More specifically, the different features described in the indigenous breeds indicate to scientists and policy makers the importance to invest in these AnGR for an optimal development of the livestock sector in Benin and in West Africa [57,58]. In addition, the study argues against systematic introduction of exotic cattle breeds in African countries with subsequent indiscriminate crossbreeding.

The loci of economic and ecological significance found under selection or in association with morphometric traits can be targeted in breeding programs aiming at enhancing animal productivity [32,59]. Ducrocq et al [60] recently illustrated the use of genomic information to improve livestock production in developing countries. Moreover, examples from Eastern and Southern African countries (Kenya, Ethiopia) evidence the importance of genetic studies (GWAS, selection signatures, copy number variation) in supporting strategies for improving animal genetic resources [34,40,61,62]. Breeding programs in West Africa are to date very rare in comparison with Eastern and Southern African regions (see [62–64]), and initiatives are expected to change this situation. Genomic approaches support not only the implementation of rapid and suitable breeding programs in an existing breed, but they can also be used to identify or to develop appropriate breeds for a given production environment through accurate selection and optimized crossbreeding [62]. The presence of causative variants underlying adaptation in the Beninese indigenous crossbreeds concurs with previous studies, which described crossbreeding as an effective strategy to enhance both economical and adaptive features in African breeds [33,37]. However, it is important to identify the optimal crossbreeding rate and preserve original indigenous breeds, in order to sustain crossbreeding programs in developing countries [62,65]. For this purpose, the current findings should be consolidated with additional investigations in the Beninese and West African cattle breeds.

3.4. Research Perspectives for the Sustainable Promotion and Improvement of the West African Indigenous Cattle Genetic Resources

As shown in this study, (West) African cattle genetic resources are relatively less addressed by genetic studies, and many aspects related to their diversity are poorly investigated and their potentials still remain largely untapped. The promising results of this study encourage further studies to genetically characterize the local cattle genetic resources at the national (in Benin) and regional (in West Africa) levels.

3.4.1. Enhance Knowledge on Genetic Diversity of Cattle Breeds in Benin

In Benin, there is a need to have an up-to-date cartography of breed distribution in the different agro-pastoral zones. The increasing settlements of Fulani pastoralists in Beninese pastoral regions have resulted in the spread of Zebu cattle and their crossbreeding with indigenous taurine breeds throughout the country [21,66,67]. For instance, the White Fulani, Red Fulani, Nigerian Fulani (Djeli), Gudali and Azawak are regularly reported in Benin, but no valid information of their geographic distribution and demography exists [68]. The expansion of crossbreed animals has also been approved in this study (Publication 2). Moreover, the hybrid animals resulting from crossbreeding with the Girolando have never been genetically investigated. Genetic diversity analyses should therefore be extended to the Zebu breeds and the different crossbreeds in Benin. In this regard, further pastoral regions unaddressed by the current sampling should be considered. Indeed, several regions sharing borders with neighboring countries and hosting major transhumance corridors for introduction of pastoralists and Zebu breeds in Benin remain non-addressed by genetic studies. These pastoral zones include Ketou (near Nigeria), Savalou (near Togo), Porga (near Burkina Faso) and Malanville (near Niger) [4,5,66]. Indeed, Malanville is reported with an important population of Gudali animals and crossbreed, whereas the W Biosphere Reserve bordering Burkina Faso and Niger in North-Benin is also described for the diversity of Zebu and crossbreed cattle [21,69]. In addition, the destinations of transboundary transhumant herders, such as Agonli and Gogounou are worth investigating for genetic cattle diversity, considering the increasing pastoral activities and cattle markets in these zones [66,70]. Also, the coastal regions in southern Benin are reported for noticeable Zebu introgression [67]. The regions of Agonli and the coastal areas are important because they represent the original belt of the indigenous Lagune cattle [71]. An appropriate evaluation of the genetic diversity may help to evaluate further crossbreeding risk of the breed as found in this study (see Publication 2). Overall, further sampling for the analyses of cattle genetic diversity in Benin should consider the ongoing changes in pastoral areas with the various sociological (e.g. transhumance), economical (e.g. animal trades) and environmental factors [5].

3.4.2. Genetic Association Studies with Functional and Economic Traits

An adequate management of cattle genetic resources in Benin also requires further evaluation of breed performances, and their association with environmental and genetic factors. The GWAS approach, as applied in this study, should target further Beninese cattle populations (previously described) and animal traits. Feed efficiency and its genetic background in the Beninese cattle breeds may be associated with animal performances (see [47,72,73]). The pleiotropic genes simultaneously controlling growth and morphometric traits as demonstrated in this study, as well as the moderate to high genetic correlations between the two trait categories reported in previous studies [74,75] indicate that growth performances and their genetic architecture can be accurately evaluated using morphometric traits. In addition, live body weight can be predicted from morphometric traits using deterministic equations [76]. However, to reach a higher prediction accuracy, the parameters included in the equations should be updated according to characteristics of Beninese indigenous cattle breeds. In addition, health traits partially reflect the adaption and impact production traits in cattle [1]. König and May [30] described several biomarkers and phenotyping strategies for the genetic evaluation of animal resistance and tolerance to disease. For instance, faecal egg counts (FEC) may be applied using affordable and/or movable equipment in Benin [77,78]. Similar affordable methods (e.g. antigen test) are effective for the diagnosis of trypanosomiasis under field conditions [79,80]. Such techniques will help to investigate the prevalence of several parasitic diseases and the genetic background of immune response mechanisms in the Beninese local breeds. Moreover, the high polymorphism degree in milk protein genes in the investigated breeds suggests additional analyses to characterize milk protein genes and their association with milk performance (including milk yield and quality) in GWAS. Besides, quantitative genetic studies should be performed to explore the genetic correlations between the milk quality and quantity in Beninese cattle populations. For this purpose, milk composition can be analyzed under field conditions in Benin using affordable and sufficiently accurate tools. The Publication 4 of this study described several other methodologies to investigate milk protein polymorphisms and milk performances. Furthermore, additional tools and approaches to enhance the collection of phenotypic, genotypic and environmental data and genetic analyses in the Beninese cattle are discussed in Publication 1. The availability of sufficient data is important to apply more advanced genomic methods including genotype by environment interaction (G × E) analyses [81]. The latter approach is a major tool to investigate genetic and environmental factors affecting animal adaptive and productive performances [81].

3.4.3. Collaborative Studies to investigate Animal Genetic Resources in West Africa

The high genetic proximity of the Beninese indigenous cattle breeds with other West African cattle breeds (e.g. the Baoulé) are confirmed in this study (Publication 2). Further, several Zebu cattle in Benin originate from, or are distributed in other West African countries (see Publication 1). Consequently, the different genetic analyses described above should be extended to the West African region. Such studies would be more effective, as they would provide a wider overview of the diversity of genetic resources in West African cattle, and their adaptive and productive features. For instance, Ibeagha-Awemu [82] previouly reported a large genetic diversity and relationships among West and Central African cattle breeds. However, the actual origin or genetic characteristics of some West African cattle breeds (e.g. the Djeli or Nigerian Fulani) are still unclear [22,83]. Selection signature analysis, GWAS or G × E analyses, targeting diverse cattle breeds from different West African countries, will be valuable to contrast the genetic pattern of the breeds with diverse production environments and socio-economic factors. Recent studies on the cattle genetic resources in Burkina-Faso provided interesting results, in line with the current study [20,43]. In addition, these studies suggest the existence of common objectives and expertise in the West African region. Therefore, collaborative research is expected for sharing knowledge and establishing regional breeding programs, which will be more economically efficient, and valuable for the West African livestock production [64].

3.5. **Significance of the study for the improvement of worldwide livestock breeding**

Although the current study focused on local West-African cattle breeds, the methodologies and findings are significantly valuable for the improvement of the global livestock breeding sector, including developed countries (like in Europe and North- America). Indeed, the main challenges affecting livestock production in Benin and West-Africa (i.e., climate change, scarcity of land and water resources, high demand of livestock products) are expected to intensify in other regions of the world, and new global breeding strategies are urgently required [84,85]. In this regard, Publication 5 described the increasing interests of farmers in developed countries (e.g. Germany, Austria) for durable alternatives toward sustainable livestock production. Similarly, researchers are increasingly suggesting more emphasis on animal adaptive features (resistance to disease, tolerance to heat stress, tolerance to feed and water scarcity or feed efficiency) [84,86], which have been largely discussed in this study. These observations imply that the close connection between genomic regions associated with adaptive and productive traits (as observed in the Publications 2 and 3) should be similarly considered for genomic selection in commercial breeds (e.g. Holstein Friesian). Moreover, the use of morphometric traits to estimate genetic parameters in this study represents an important example for phenotypic trait recording and genetic evaluation of animals. These measurements, in contrast to the subjective scores for conformation traits which are commonly used in European countries, are more accurate and imply larger heritabilities [87,88]. Furthermore, the rather uncommon multi-breed approach for genetic parameter estimations and genome wide association analyses contributed to moderate heritabilities and genetic variances, as well as common causative loci across breeds in this study [89,90]. The results confirm the potential of the multi-breed approach for genetic studies addressing composite breeds or crossbreeds. Due to the fact that composite breeds are increasingly being developed over the world and several regions are characterized by large diversity of animal breeds [65], multi-breed GWAS is an interesting approach to consider additional breeds with small population size [90]. In addition, the latter approach supports the establishment of collaborative genetic and genomic studies including different environmental or production factors. Finally, the genome-wide scan using temporal subpopulations and applying the XP-EHH methodology with adjusted *p-values* was able to capture genomic regions under selection, while reducing false positives and bias due to the uncertainty of ancestral base populations. Indeed, population structure and demographic history are major factors affecting selection signature studies [32]. In consequence, the current findings will certainly stimulate further improvements in statistical methodologies and experimental designs for livestock genetic studies.

3.6. Main findings of this study

The current study generated several essential outputs, which can be summarized as follows:

- A holistic approach (considering farmers breeding objectives, sufficient phenotypic and genomic information) is required to address the improvement and sustainable use of the large diversity of animal genetic resources in Benin.
- The Beninese indigenous cattle breeds (Somba, Lagune, Dahomey, Borgou, Pabli) presented closer genetic relationships to West African cattle, in contrast to European or Asian breeds.
- The Lagune breed was confirmed as the ancestor of the Dahomey cattle kept in Europe.
- High genomic inbreeding coefficients were estimated in the Dahomey cattle (kept in Europe) in comparison to the indigenous cattle breeds kept in Benin (Somba, Lagune Borgou, Pabli).

- The Borgou population displayed higher admixture effects in comparison with other Beninese indigenous breeds, especially the taurine (Somba, Lagune).
- Several genomic regions affected by selection pressures were identified in the Beninese indigenous cattle breeds. They hosted QTL and genes related to both adaptive and productive traits.
- Genetic parameters (heritabilities, phenotypic and genetic correlations) for morphometric traits were moderate to large in the Beninese cattle breeds.
- Multi-breed GWAS were effective to investigate the genomic architecture of functional traits in diversified animal populations, like the Beninese indigenous cattle breeds.
- Genomic regions associated with morphometric traits in the Beninese indigenous cattle were involved in growth and carcass as well as stress, immune response and feed efficiency traits.
- Strong genomic associations between adaptive and productive features were observed in the Beninese indigenous cattle breeds.
- Milk protein genes (*CSN1S1*, *CSN2, CSN1S2*, *CSN3*, *LALBA*, and *LGB)* presented a large genetic variability with several new SNP and InDel) in the Beninese indigenous cattle breeds.
- Identified polymorphisms in milk protein genes induced novel milk protein variants in the Beninese indigenous cattle breeds.
- The genetic polymorphisms affected microRNA and transcription factor binding sites.
- The major casein haplotypes for *CSN1S1*-*CSN2*-*CSN1S2*-*CSN3* in the Beninese indigenous cattle breeds included B-A1-A-B, B-A1-A-A and C-A2-A-B.
- The Dahomey cattle were increasingly attractive to European farmers, because of its suitability for landscape conservation and easy management.
- The interests for small-sized cattle breeds and their importance for sustainable livestock production were valuable incentives (to researchers and policy makers) to promote and conserve the local Beninese and African cattle breeds.

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List of Further Publications during the Doctoral Study

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Formal Declaration

I declare that the dissertation here submitted is entirely my own work, written without any illegitimate help by any third party and solely with materials as indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At all times during the investigations carried out by me and described in the dissertation, I have followed the principles of good scientific practice as defined in the "Statutes of the Justus Liebig University Gießen for the Safeguarding of Good Scientific Practice".

Gießen,

Sèyi Fridaïus Ulrich Sédric Vanvanhossou

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