Isolation of a pentacyclic triterpenoid from *Sceletium tortuosum*

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Abstract

Traditionally, extracts from Sceletium tortuosum have been used, both as a medicine as well as for social and spiritual purposes. The genus is distributed in the south-western parts of South Africa. Methanolic extracts of S. tortuosum were prepared and fractionated using column chromatography. Indole alkaloids have been reported from S. tortuosum with mesembrine as the most abundant alkaloid and mesembrenone, 4'-odemethylmesembrenol and tortuosamine also present. A triterpenoid, novel to this genus was isolated and reported for the first time. NMR and HPLC-MS/MS analyses were used to confirm the structure of the compound.

Key words: Sceletium tortuosum, alkaloids, triterpenoid, obtusalin.

Introduction

The genus Sceletium is classified under the family Mesembryanthemaceae (Aizoaceae) and belongs to the sub-family Mesembryanthemoideae (Patnala and Kanfer, 2009; Smith et al., 1996). The genus name is derived from the word 'sceletus' which means 'skeleton', referring to the prominent vein-like lines, which are easily visible in the old, dry and withered leaves (Figure 1) (Gericke and Viljoen, 2008). The word Mesembryanthemum originates from the name 'midday flower', which refers to the opening of the flowers around noon (as shown in Figure 2). Plants that belong to the Mesembryanthemaceae family are known by most South Africans as vygies (Chesselet et al., 2002)

Figure 1: Characteristic skeletonised appearance of old leaves (Gericke and Viljoen, 2008).

Figure 2: Represents that decumbent habit and succulent leaves of *Sceletium* spp. (Gericke and Viljoen, 2008).

The general application of Sceletium (Aizoaceae, subfamily Mesembryanthemoideae) had been revised by various authors since the genus was established in 1925 by N.E. Brown (Gericke and Viljoen, 2008). This group of plants is characterised by the skeletonised leaf venation pattern visible in dried leaves. In 1986, Bittrich argued for a broader application of Phyllobolus which included Sceletium as one of five subgenera (Gericke and Viljoen, 2008). Since Gerbaulet (1996) was unable to find a synapomorphy (a unique derived character) for Bittrich's broad concept of Phyllobolus, she reinstated Sceletium as a genus (Gericke and Viljoen, 2008). Species of this genus are distinguished on the basis of vegetative, flower, fruit and seed characteristics. Some species are reduced to synonymy including S. joubertii L. Bol., and S. namaquense L. Bol. now considered part of S. tortuosum. Sceletium exhibits a climbing or decumbent habit and has characteristic succulent leaves with "bladder cells" or idioblasts.

The genus Sceletium is distributed in the south-western parts of South Africa and has an affinity for arid environments. It is amongst taxa that have been extensively researched in the past few decades. Traditionally, plants of this genus have been used to relieve thirst and hunger, to combat fatigue, as medicines and for social and spiritual purposes by San hunter-gatherers and Khoi pastoralists (Gericke and Viljoen, 2008). South Africa can benefit from the scientific evaluation of this indigenous plant and its knowledge base. However; the chemistry and pharmacology of many medicinal plants, such as Sceletium tortuosum, have not yet been thoroughly investigated (Harvey, 2000). It is believed that the phytochemical exploration of the genus Sceletium commenced in 1898 when Meiring isolated a crude alkaloid mixture from S. tortuosum. This was followed by the work of Zwicky in 1914 that isolated several alkaloids including mesembrine and mesembrenine (Gericke and Viljoen, 2008). The number of Sceletium species within the Aizoaceae family that have been examined for the presence of alkaloids has been restricted by their geographical inaccessibility. Within the Sceletium genus, a number of alkaloids are produced which mainly belong to the crinane class of compounds (Jeffs et al., 1982), but no compound belonging to the triterpene class has been reported to have been isolated from Sceletium.

2. Material and Methods

2.1 Plant collection

Nineteen kg of Sceletium whole plant material was harvested on 11 – 12 June 2009 under sunny and dry conditions at Kamieskroon in the Northern Cape of South Africa. The plant material was supplied to the Council for Scientific and Industrial Research (CSIR) for this study by Enterprise Creation for Development (ECD). Plant material was collected by an independent contractor who practiced standard operating procedures during harvesting/handling, storage and transportation. This study required harvesting of whole plant material by the supplier. The plant material was positively identified and confirmed to be Sceletium tortuosum N.E. Br. by the South African National Biodiversity Institute (SANBI), batch number: 10025 (Genspec No. ECDMP-100 22).

2.2 Plant preparation and extraction

About 6.3 kg of wet plant material was placed in a 60 oC oven to dry. The dry plant material was not ground to a fine powder, but was broken and crushed into smaller pieces. From that, 3.15 kg was transferred to a 1000 ml glass beaker. Two large magnetic stirrers and sufficient methanol (MeOH) solvent, enough to cover the plant material was added. The beaker was covered tightly with a piece of aluminium foil before being placed on a stirring heating plate and heated at 40 oC for 2 days (48 hrs). A mercury thermometer was used to monitor the temperature throughout the extraction process. After extraction, the contents were mixed thoroughly and then filtered through filter paper into a 1000 ml conical flask connected to a vacuum pump. The extract was filtered twice to remove any debris remaining in the suspension. The MeOH was evaporated at 45 oC using a GeneVac® Personal evaporator. The crude extract was then stored in a dark, cold room at 4 oC.

2.3 Compound isolation and purification

Obtusalin was isolated from the methanol extract of S. tortuosum using Preparative Layer Chromatography (PLC) with a solvent system containing hexane, ethyl acetate and triethylamine [Hex: EtOAc: Et3N (6:4:1)] as eluant. The plate was run twice to improve separation between compounds. A very thick band was observed under UV light and used for further purified using PLC with Hex: EtAc: Et3N (6:4:1) as solvent system. Ultimately, 2 mg of obtusalin was isolated. NMR (600 MHz, Varian,) was used to confirm the structure, using 1H, 13C, COSY, DEPT, HSQCAD and HMBCAD protocols to generate chemical shift data for subsequent comparison with that found in the literature.

3 Results and discussion

The methanol crude extract of S. tortuosum was fractionated and several compounds were isolated, purified and identified. These compounds were confirmed to be alkaloids that have commonly been reported in the extraction of S. tortuosum; namely, mesembrine, Δ4 mesembrenone, Δ7 mesembrenone (mesembranol) and epimesmbrine. An unexpected finding which later became apparent, was that these compounds degrade over a period of time, verified by the degradation of mesembrine. Consequently; the compounds had to be re-isolated, with more attention being paid to chemical instability problems. A separate High Performance Liquid Chromatography (HPLC) study was undertaken to evaluate the stability and determine if there were gradual changes occurring within the extracts while in storage. Mesembrine and Δ7 mesembrenone were re-isolated, with some measure of stability, but the other two compounds had degraded to such an extent within the extract that they could not be re-isolated. In addition to these compounds, pinitol and sucrose were also isolated from the plant (Setshedi, 2014).

A pentacyclic triterpenoid confirmed as obtusalin (Figure 3) was isolated for the first time from the methanol extract of S. tortuosum. Triterpenoids are generally very stable and, unlike the alkaloids of this plant material, do not decompose, but were found in low concentrations. Triterpenoids are commonly found in most plants and are produced by arrangements of squalen epoxide in a chair-chair-chair-boat arrangement subsequently followed by condensation (Patočka, 2003). These compounds are isopentenoids composed of thirty carbon atoms and may possess acyclic, mono, di-, tri-, tetra- or pentacyclic carbon skeletons. Pentacyclic triterpenoids are dominant constituents of this class and have been widely investigated (Mahato and Kundu, 1994). Obtusalin is a pentacyclic triterpene belonging to the lupane class of compounds (Zheng et al., 2010; Siddiqui et al., 1989) that was first isolated by Siddiqui and colleagues in 1989 from the leaves of Plumeria obtusa. Obtusalin forms part of is the few naturally occurring pentacyclic triterpenoids possessing a C-27-hydroxyl group in conjunction with a double bond at C-12 in the lupine chain of triterpenoids (Begum et al., 1994 and Siddiqui et al., 1989). Pentacyclic triterpenes are reported to possess a wide spectrum of biological activities, where some may be used as medicines (Patočka, 2003).

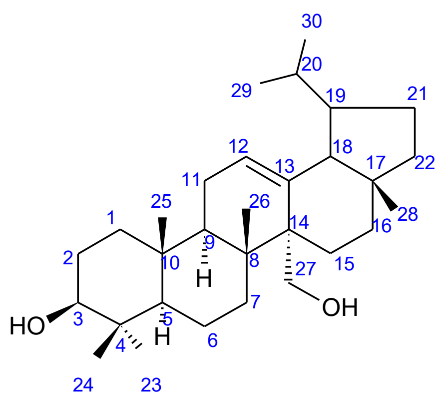

Figure 3: Structure of Compound 1

Table 1: [1]H NMR spectral data of compound 1 ($CDCl_3$) in comparison to that found in literature ($CDCl_3$) (Siddiqui et al., 1989)

Compound 1

Proton Number	***δ*H (*J* in Hz)(isolated)**	***δ*[13]H(*J* in Hz) (Literature)**
3***α***	3.16 (br*d*, *J* = 12.69)	3.21 (*dd*, *J* = 10.8; 4.9)
5***α***	0.68 (br*t*, *J* = 12.1)	0.72 (*dd*, *J* = 11.6; 1.5)
9	1.54 (m)	1.54 (*dd*, *J* = 10.0, 3.4)
11***α***	1.85 (br*dd*, *J* = 8.8, 6.4)	1.84 (*ddd*, *J* = 13.2, 3.6, 3.4)
11***β***	1.63 (m)	1.61 (*ddd*, *J* = 13.2, 10.0, 3.6)
12	5.07 (*brs*)	5.13 (*t*, 3.6)
23	0.94 (*s*)	1.01 (*s*)

ISSN: 2707 – 2843 (Print) 2707 -2851 (Online)

24	0.93 (s)	0.98 (*s*)
25	0.72 (s)	0.78 (*s*)
26	0.88 (s)	0.94 (*s*)
27a	3.46 (brd, *J* = 11.2)	3.52 (*d*, *J* = 10.9)
27b	3.14 (*brd, 11.2*)	3.18 (*d*,10.9)
28	1.03 (*s*)	1.10 (*s*)
29/30	0.87 (*brd*, *J* = 5.2)	0.93 (*d*, *J* = 5.8)
30/29	0.75 (*brd, J* = 7.0)	0.80 (*d*, *J* = 5.9)

Table 2: ^{13}C NMR spectral data of compound 1 ($CDCl_3$) in comparison to that found in literature ($CDCl_3$) (Siddiqui *et al*., 1989 and Begum *et al*., 1994)

Compound 1

Carbon Number	**δ^{13}C (Isolated)**	**δ^{13}C (Literature)**
1	38.8	38.8
2	27.2	27.3
3	79.0	79.1
4	38.0	38.0
5	55.1	55.2
6	18.3	18.4
7	32.8	32.9
8	40.0	40.1
9	47.6	47.7
10	36.9	36.9
11	23.4	23..4
12	125.0	125.1
13	138.7	138.8
14	42.0	42.1
15	23.4	23.4
16	26.0	26.0
17	38.8	38.8
18	54.0	54.0
19	39.4	39.5

20	39.3	39.4
21	30.6	30.7
22	35.2	35.2
23	28.1	28.2
24	16.8	16.8
25	15.6	15.6
26	15.7	15.7
27	69.9	69.9
28	23.3	23.3
29	21.3	21.3
30	17.3	17.3

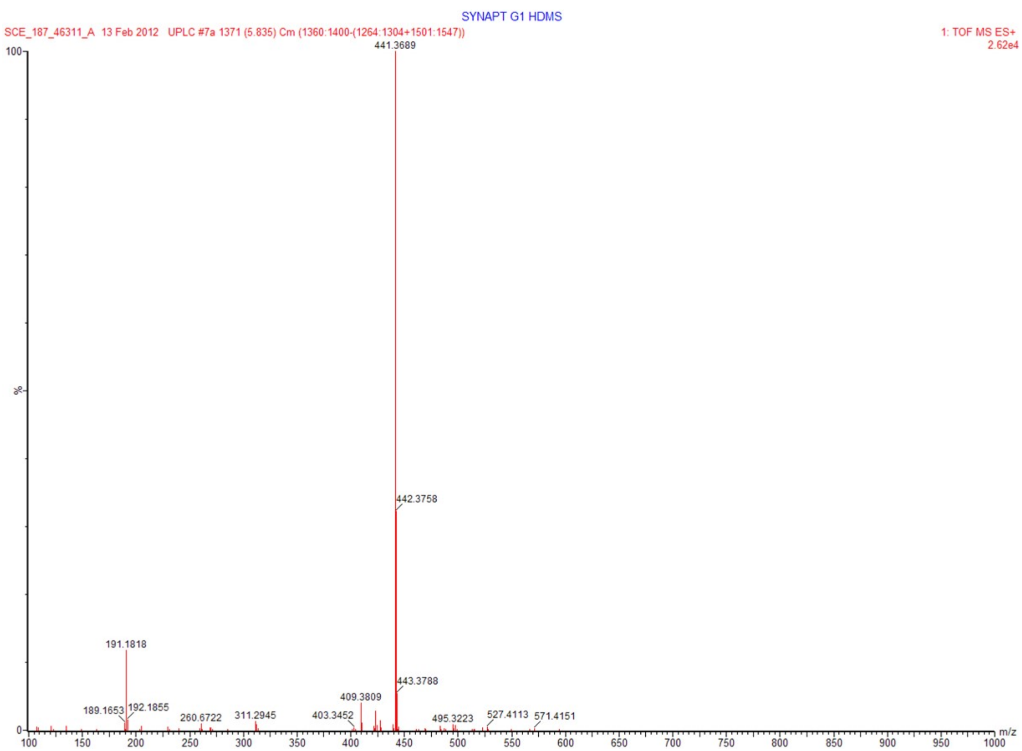

Figure 4.: HRTOFMS (ESI^+) chromatogram of compound 1

The HRTOFMS (ESI+) spectrum (Figure 4) of compound 1 showed a pseudo-molecular ion signal [M]+ at m/z 441.3689 which corresponds to the molecular formula C30H50O2. There are significant fragments as indicated in Figure 5. This data strongly indicates that the compound is of a lup-12-ene type. 1H NMR, 13C NMR and MS spectral data of compound 1 are in agreement with those reported in literature for obtusalin (Siddiqui et al., 1989 and Begum et al., 1994). One olefinic proton was observed in the proton NMR spectrum (Table 1) and resonated at δH 5.07, while two secondary methyl groups resonated at δH 0.87 (J= 5.2 Hz) and δH 0.75 (J= 7.0 Hz), five three-proton singlets indicating tertiary methyls that resonated at δ 0.94, 0.93, 0.72, 0.88 and 1.03. The 13C NMR spectrum (Table 2) showed the presence of 30 carbon signals: olefinic carbons at δC13 125.0 and 138.7; oxygenated carbons at δ

79.0 and 69.9 and seven methyls at δC13 15.6, 15.7, 16.8, 17.3, 21.3, 23.3 and 28.1. Accordingly, the structure of compound 1 was assigned as obstusalin.

4 Conclusion

Sceletium species have been shown to contain indole alkaloids with mesembrine reported as the most abundant alkaloid in S. tortuosum. A pentacyclic triterpenoid, novel to this species as well as the genus was isolated and reported for the first time. NMR and HPLC-MS/MS analyses were used to confirm the structure of the compound. Pentacyclic triterpenes are reported to possess a wide spectrum of biological activities, with some used as medicines.

5 Acknowledgments

We thank the Department of Science and Technology for their financial support.

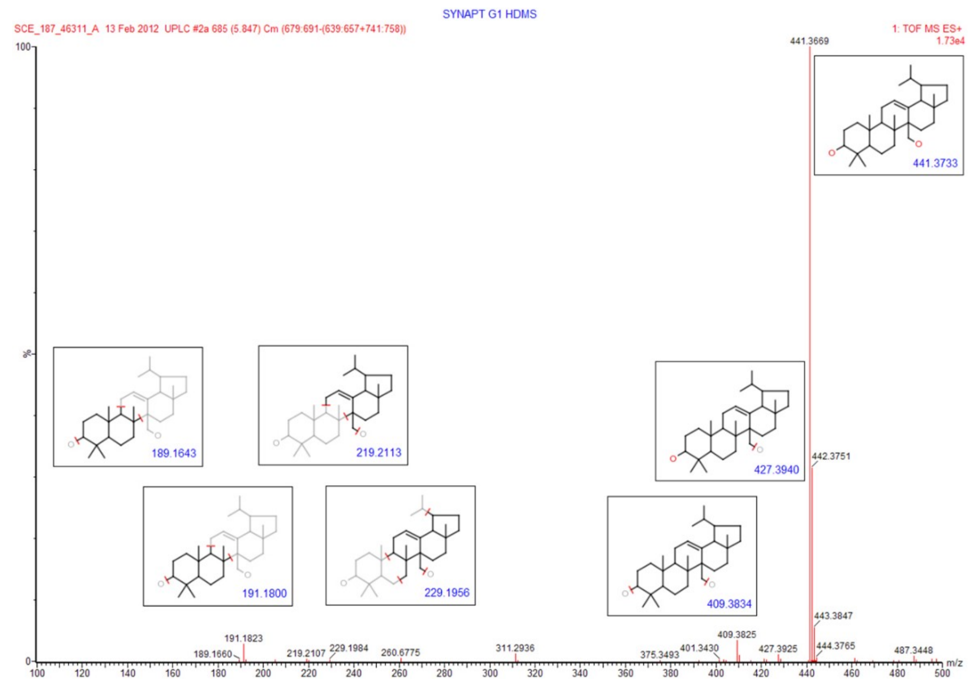

Figure 5: HRTPFMS (ESI^{+}) fragmentation of compound 1. Showing peak fragmentation correlating to those significant fragments which strongly suggests that the compound is of a lup-12-ene type.

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Factors affecting the global diffusion of an African animal genetic resource: the case study of the *Cameroon Blackbelly* sheep

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Abstract

Sheep farming is a vital component in the food, economic and socio-cultural security of many countries around the world. Sheep are present in most countries of the world, proving their adaptation capacities to different climatic conditions and agro-ecological zones in different countries. Because of these capabilities, they have been widely disseminated in order to enhance their genetic potential. Several elements have contributed to the diversification and dissemination of this biodiversity, including the human slave trade waves. During these movements, Cameroon ovine biodiversity has been exported and distributed throughout the world, particularly the Blackbelly sheep breed. Within the framework of the World Action Plan focusing on the characterization of native biodiversity, it is important to trace the dispersal routes and models of this particular breed across the world in order to demonstrate the impact of slave trade in the dissemination of animal material, but also to collect information on the ways of adaptation and exploitation of the Blackbelly sheep around the world. This could enable the conservation and better improvement of this genetic resource in Cameroon for the benefit of population. From publications collected on the matter around the world, it appears that slave trade and the triangular trades were the main factors of global diffusion of the Cameroon Blackbelly sheep. The Blackbelly has been spread in two continents: America and Europe, but its main settling area was the Caribbean. The Barbaric Island was the main point of adaptation of this breed hence the name *Barbados Blackbelly* from which several countries have refueled. The Blackbelly sheep has been adopted as an official breed after improvement in 4 countries: Barbaric Island (*Barbados Blackbelly*), the United States (*American Blackbelly*), Germany (*Kamerun Schafe*), and in Martinique (*Martinik Sheep*). All these varieties share some specific features that have remained very close to that of the sheep of Cameroon notably: the brown coat color with a blackbelly, resistance to diseases (trypano-tolerant), hardy, and prolific. Worldwide, the Blackbelly sheep is exploited for meat, leather, prestige, ecotourism and milk in some extent. The current state of information on Blackbelly in Cameroon is limited to descriptive study and some phenotypic assessment. In-depth studies on its mode of exploitation and its molecular genetics will certainly allow better understanding of this breed to plan for methods of conservation and sustainable improvement.

Key words: *Blackbelly sheep, colonization, international trade, biodiversity, Cameroon*

Introduction

Sheep or *Ovis aries* Linnaeus 1758 is one of the oldest domestic animals. It is widespread throughout the world, with high numbers, demonstrating the ability to adapt to different climates and universal interest (Meyer et al., 2003). The sheep is one of the first species domesticated by humans after the dog and goat, around 9000 - 8500 BC. The domestication of sheep took place in the region of the "fertile crescent" (Turkey, Syria, Iraq, present day Iran) (Meyer et al., 2003). Sheep from Africa and Europe are distributed from this center (Lauvergne, 1979). The sheep appeared for the first time in Europe in 6300 BC to Argissa-Magula. It arrived in Western Europe a few centuries later, by land and by sea. A second wave of sheep arrived in Western Europe during the second and the third century. These animals were larger and had wool fleece. The sheep was already present in North Africa, entering through Egypt around 5000 BC. In Africa, there are trypano-tolerant sheep breeds, indicating the ancient presence of this species locally (Mason, 1951; Meyer et al., 2003). These are smaller sheep adapted to the ecological conditions of West Africa, hence the name "West African dwarf sheep". With the movements of humans and animals, several animal species have moved from one area to another. These movements have been at the origin of several sheep genetic breeds in the world.

This paper finds traces of several very old breeds in areas that are not their zone of origin, for the particular case of Blackbelly (from Cameroon) their traces can be found in America, the Caribbean and even in Europe. Genetic characterization has for some years been the method of describing and classifying livestock breeds using measures of genetic distances between populations (Cavalli-Sforza and Edwards, 1967; Nei, 1972; Nei et al., 1983). Indeed, for sheep, Lauvergne (1979) explains that visible markers could be used to measure quite simply the degree of archaism of a sheep population: composition, coloration and molting of the fleece, type of horn, conformation of the head (chamfer, ears), tail shape. This facilitates the description of sheep genetic diversity in the world. Nijman et al., (2003) supports that mtDNA can also provide a quick way to detect hybridization between farm species and wild sub-species. Of recent, microsatellites became prime markers for the study of diversity (Ruane 1999, Sunnucks 2001) because of their co-dominant nature, ease of amplification and hypervariability while SNPs are becoming more popularized. The blood groups and biochemical polymorphisms of domestic animals have been studied since the early 1960s and have since been used to compare races and study the relationships between them (Grosclaude et al., 1990).

It happens that 5 main breeds were described in Cameroon and are the subject of a summary knowledge of their characteristics: Doutressoule (1947) described the Djallonke, Bardoux (1986) described the Kirdi breed, Dumas (1977) worked on the Peuhl and Uda breeds, Manjeli (1998) described the Blackbelly breed.

Origin and description of the Cameroon Blackbelly sheep

The characterization of animal species consists in the first phase in a classification and description of animal units called races (Gizaw, 2008). The genetics of African pastoralism is controversial and often very underdeveloped (Hanotte et al., 2002). Djallonké sheep include all the most common trypano-tolerant sheep populations in tsetse-infested areas of West and Central Africa; south of the 14th° parallel (FAO, 1992). These sheep would come from Fouta Djallon in Guinea (hence the origin of the name Djallonke) and would have for ancestor, the Egyptian sheep *Ovis longipes* (Devendra and McLeroy, 1982). The most commonly encountered coat patterns are black, black-legged and more rarely white, red, red-legged (Figure 1). In certain regions (particularly in the East of Cameroon), there are animals with a particular tawny coat with belly and legs colored black, which gives them the name of *Blackbelly* in English (Vallerand and Branckaert, 1975). The Blackbelly sheep is a breed with a fine (smooth) tail, the head is small with a straight profile. The ears are small and slightly drooping (Manjeli et al., 1991). It can reach 55 to 65 cm at the withers. This sub-race is genetically stable and the animals have a more advantageous external appearance. This impression is confirmed by their average weight, which in adult females of this sub-breed exceeds 28 kg (Vallerand and Branckaert, 1975). In Cameroon the Blackbelly sheep is used only for the production of meat, the male can reach 25 to 35kg. Nevertheless, the production of Cameroon's Blackbelly sheep milk is appreciable, ranging from 2.95 to 3.52 liters per week (Manjeli et al., 1991).

Figure 1: Cameroon Blackbelly young ram

Spreading factors and exploitation of Blackbelly sheep

The Blackbelly sheep by its qualities of adaptative production traits has been widely distributed in the world for a hair sheep. This has undoubtedly been favored by several factors such as triangular trade and the slave trade. This demonstrates that the method and chronology of dispersion of African animal breeds also have a non-nuclear cause (Hanotte et al., 2002). These two movements of the western colonial era have had a significant impact on the distribution of animal material, and more specifically on dwarf sheep in West Africa (Naves et al., 2011). One of the determining factors for the spread of Blackbelly sheep in the Caribbean and some Latin American countries were triangular trade with West African ports, simultaneously with human trafficking (Naves Met al., 2011). It resulted in recurrent introductions of domestic animals from West Africa, between the sixteenth century and the beginning of the nineteenth century (Maillard and Maillard, 1998). For instance, the analysis of mitochondrial DNA of some bovine genotypes in the Caribbean and America has demonstrated their African origin. In Asian chickens, nine different clades of mtDNA have been identified (Liu et al., 2006) that suggest multiple origins in South and Southeast Asia. These results indicate that the knowledge about farm animals' domestication and genetic diversity is still largely incomplete. Therefore, it is established that there is a strong genetic component of African origin in the Caribbean countries' sheep breeds (*Blackbelly* or *Pelibuey*), in the Creole goats of the Antilles (Pépin, 1994), and in the Creole cattle of Guadalupe (Miretti et al., 2004;

Naves and 2011). Exchanges have also taken place between the Caribbean islands and the American continent, in the North as well as South (Maillard and Maillard 1998; Lucero et al., 2010).

Expansion to the Caribbean and America (South and North)

The Blackbelly sheep has had a large dispersal in the Caribbean and mainly in the Barbarian Island (Shelton et al., 1990). During the period of the slave trade, the triangular trade allowed the transit of goods between America, Europe and Africa. These movements across the Atlantic Ocean included several Caribbean countries (Cuba, Dominica, Jamaica, Trinidad and Tobago, Martinique, Barbados etc.). The ships from Africa transported slaves but also animals from Africa. This movement allowed the transport of African sheep breeds to the Caribbean. Historic research demonstrated that the *Furry sheep* in the Caribbean is of African origins, namely from Nigeria, Angola and Cameroon during the period of slave trade (Mason, 1980).

The Barbados

Barbados could be considered as the main area of installation and adaptation of Blackbelly sheep from Africa (later mentioned as *Cameroonian sheep*). According to the BBSAI (2011), the Blackbelly arrived in the Barbados Island during the period of colonization of the island by the Europeans. Combs (1979) has established that during the first quarter of the century of colonization, a batch of Blackbelly sheep was set up on the island following British colonization in 1627. This sheep adapted to this climate during the exchanges of the slave trade has medium and horizontal ears. It is called Barbados blackbelly with reference to Barbados which is at the origin of its expansion (Shelton et al., 1990). The pattern of Blackbelly color, which seems to be simply inherited, is found in the current populations of *Djallonke* type sheep in West Africa. This observation, and several other features that the breed shares with the *West African* sheep, including the shape and size of the ear, of course, the hair (Figure 2), indicates a West African origin for the *Barbados Blackbelly* sheep breed. However, the latter has two distinctive features, namely: a larger average size of all "West African" populations, and higher prolificacy (Fitzhugh and Bradford, 1980). It is a larger sheep than Cameroon sheep and is generally used for meat production. Ewes raised from 1972 to 1977 at three locations in Barbados produced 1079 litters of milk, with 2194 lambs, an average of 2.03 lambs per litter, and at an average lambing interval of 8.48 months. Rastogi et al (1980) observed 26.8% single birth, 47.3% twins, 22.1% triplets, 3.4% quadruplets and 0.4% quintuplets.

Figure 2: Barbados Blackbelly ram

Cuba

According to Mason (1980), the Cuban sheep apparently also came from Africa. It is estimated that about 75% of sheep in Cuba are hair breeds originally called *Pelobuey Carnero* (that is, beef-haired sheep) that has been shortened to *Pelibuey*. These sheep are also called *Criollo*, but they differ from the real *Criollo* of the mainland of Latin America, which are wool sheep of the Churro breed of Spain (Shelton et al., 1990). *Pelibuey* sheep have a variety of colors, including brown (or red), cream whitish and white, black, and more. The first three colors are the most common and are colors that would be inherited from their African origins. *Pelibuey* sheep on state farms are either red or white. Red animals, which are believed to be physically stronger, are preferred. In addition, the red color is dominant in the *Pelibuey*, but not compared to the white types in the European breeds.

Dominican Republic

There are haired sheep in the Dominican Republic. According to Mason (1980), there are about 52,000 sheep in the Dominican Republic and 90% of them are haired sheep. There is a report of some Cuban *Pelibuey* sheep from the Dominican Republic (Mason, 1980).

Mexico

There are also traces of Blackbelly in Mexico. In fact, there is a sheep breed in Mexico called *Tabasco* or *Pelibuey* of Mexico whose origins are connected to the Blackbelly sheep of Africa. Figueiredo et al., (1990) in the analysis of sheep origin assume that the Pelibuey of Mexico was introduced from Cuba around the 1930s. In addition to the absence of horns in both sexes, it was found that coat colors patterns are close to those in Blackbelly. Zarazua and Padilha (1983), have shown that this breed is easily adapted to wet climate, with longer rainy seasons, where heat and humidity are high, similar to the humid tropics' conditions. This breed is raised mainly for meat; mature animals are 64-66cm for the height at withers, with live body weight ranging from 35 kg(female) et near 50kg (male).

Trinidad Tobago

Sheep production has been practiced in Tobago for more than 100 years. There were no native sheep in Tobago and the current sheep are descendants of the imported breeds. As early as 1909, a ram and two purebred West African ewes were imported from Barbados. It is unclear as to when Barbados first imported Blackbelly to Tobago occurred. In 1953, the breeding policy of the Tobago Government Farm was directed towards the gradual establishment of a flock of Blackbelly sheep from Barbados and in 1958 three sheep breeds were maintained there, namely: *Blackhead Persian*, *West African Dwarf* and *Barbados Blackbelly* (Fitzhugh and Bradford 1980). Later on, other imports of Barbados Blackbelly took place from the West Indies (in 1977) and Barbados (1977-78). The majority of sheep in Tobago are raised by small farmers in groups of one to five as a ready source of money or meat for a special occasion. In Trinidad Tobago farms, the Blackbelly sheep can weigh 21 to 45kg at maturity.

Colombia

Colombia has also inherited animal genetic material during the slave trade (Shelton et al., 1990). African sheep in Colombia are similar to *Pelibuey* from Mexico. The yellow or Sudan type varies from yellow to reddish-brown in color (Otero and Cruz, 1980). African sheep are also known as *Pelona* or *Camura* and have existed in Colombia for over 300 years. Some exceptional characteristics of these genotypes include adaptation to warm climates, hardiness and quality of meat. It is generally recognized that these sheep were brought from West Africa on slave ships (Shelton et al., 1990). In Colombia, sheep are generally kept in small herds of less than 50 animals grazing the less productive areas of the farms. They receive special care and derive much of their food from browsing and agro-industries by-products. Their gestation period is 151.7 days for single lambing, with lambing intervals of 213.6 days (Pastrana et al., 1983). Animals weigh 2.5 kg at birth, 15-18 kg at weaning (4 months), 35-40 kg for one year and 49 and 45kg for male and female fattened animals (12 months old). An adult male under intensive conditions can weigh up to 80kg (Otero and Cruz, 1980). Other studies show that adult females and males weighed 36.7 and 48.7 kg, with 78.0 and 84.0cm for chest girth, and height at the withers 62.9 and 68.7cm respectively (Pastrana et al., 1983). The average age at first lambing is 15-19 months. The fertility rate reaches 98%, with a prolificacy rate of 1.34.

United States

The United States have for several years given particular interest in Blackbelly sheep. Genetic analysis of modern American breeds has demonstrated a variety of origins: Europe (Campbell and Lasley, 1985; Davis et al., 1988), Africa and India (Speller et al, 2013). The Barbados Blackbelly come from crosses made from sheep with hair from East Africa. Originally from Barbados, they have extended to the entire West America. According to Combs (1979) 4 ewes and a Barbados Blackbelly ram were imported to the US in 1904 by the USDA. It is from these that the *American Barbados Blackbelly* or *American Blackbelly* sheep breed was developed (CODD, 2017). It is assumed that two types of fine (smooth) tail sheep came to America. One, without mane, probably from savannah areas in the Congo region, including northern Angola and Cameroon. These are believed to be the ancestors of the *Morada Nova* of north-eastern Brazil and other hairless sheep (Mason, 1980; Bradford and Fitzhugh, 1980). The animals that gave origin to these types of sheep were probably selected from flocks with a high frequency of brown color and its combinations. The American Blackbelly sheep type (American variant) looks more like a Cameroonian type of great size. This breed with the *Painted Desert*, the *Texas Dall*, the *Desert Sand* and the *Black Hawaiian*, is part of the "Corsicans sheep". All these breeds have the particularity of having been obtained in the last century in the United States, by crossing animals from the Barbados with Mouflons (Corsica), but also Rambouillet Merinos and Dorsets (CODD, 2017). The coat is exclusively tawny, from light to reddish brown (Figure 3). This breed is considered mostly for meat. The weight of American Blackbelly is significantly higher than that of the Cameroon type; records in mature individuals show 50 to 60kg for the ewe and up to 90-100kg ram. The prolificacy of the American Blackbelly is close to 200%, doubles or triplets are common, quadruplets are not rare and occasionally quintuplets.

Figure 3: American Blackbelly ram

The Martinique

Sheep were introduced to the West Indies from colonization and more likely from the shores of the Gulf of Guinea in Africa. They are hairy sheep, closely related to the Djallonke sheep or West African types. The Martinik sheep is selected from Barbados Blackbelly. In 1993, the Martinik sheep benefited from the establishment of a breeding program based on seven private farms grouped together in a Selection Body (Union for the Selection of Martinik Sheep) approved by the Ministry of Agriculture. Carried out in collaboration between the various partners of the genetic sector in France, it was then possible to supply the breeders with sheep of well-known breeds, and evaluated on their results obtained in control of performances (Vertueux et al., 2006). Of recent creation, the *Martinik sheep* breed includes animals of various coats, but with very similar abilities. The adult sheep weighs on average from 35 to 45 kg and the rams from 50 to 70 kg, according to the conditions of breeding. The height at the withers for ewes is around 55cm. The small ears are borne horizontally and the tail is of medium length (above the hock); fertility is 85% and productivity 1.9 (USOM, 2008). Well-fed adult *Martinik sheep* are generally strong enough not to require anthelmintic treatment, with the exception of a few ewes around lambing, with litters of two or more lambs. Recent comparisons with woolen breeds have also demonstrated higher genetic resistance to parasites in Martinik lambs, making them an interesting breed for valorizing tropical pastures (INRA, 2014).

Figure 4: Martinik ram - (USOM, 2008)

Expansion to Europe

Europe was the decision point of the spread of Blackbelly sheep to the Caribbean and America through the triangular trade of the colonial era.

Germany

Originally, from West Africa, the path that led this wild sheep breed to Europe was unusual. In Germany around the 1900s, Zoological gardens were created. For this reason, ferocious beasts have been shipped from African colonial countries amongst which were lions and leopards. As provisions for wild animals, a breed of African sheep was brought. Some sheep survived these trips and started being reared. Germany empire was established in then called *Kamerun* from where the colonial administration extracted numerous living resources to Europe where traces are still visible. The cradle of Cameroon Blackbelly sheep is East Forest (Moloundou) where a small German colony was established in late 1800s. Sheep in parks and zoos have gained great popularity and today they are mainly found in amateur breeders, because the Cameroon sheep are convenient and robust landscapers. Called "*Kamerun schafe*" (Figure 5), Cameroonian sheep experienced a very good adaptation in Germany (Ladybug-Farm, 2010). Most black sheep breeders keep their sheep as meat suppliers. The objective is the proper formation of valuable pieces of meat on the back and leg. Animals slaughtered between 5 and 8 months for about 10-16 kg of meat. The meat has a taste and appearance similar to that of game meat such as the usual lamb meat. The exquisite taste is not lost even in the slaughter of old animals.

Figure 5: Kamerun schafe ram

Other European countries

The Cameroonian sheep has also been located in several other European countries, particularly in France, England and the Netherlands. Although the mechanisms of diffusion remain very difficult to trace, the authors agree that it was favored by the trade routes favored during the period of colonization. From Combs (1983) findings, Blackbelly sheep was introduced in England and Netherlands during the first quarter of 19^{th} century. There have been some early breed associations and relatively interesting literature on this breed in Central and even eastern Europe which could have contributed to the popularization of this sheep genotype to Europe and other directions as pet or zoo animal (Mathieu, 1997).

Migration waves and spatial distribution Blackbelly sheep

Multiple movements of Blackbelly sheep occurred in several centuries, first northern to Europe and western as shown by the figure 6.

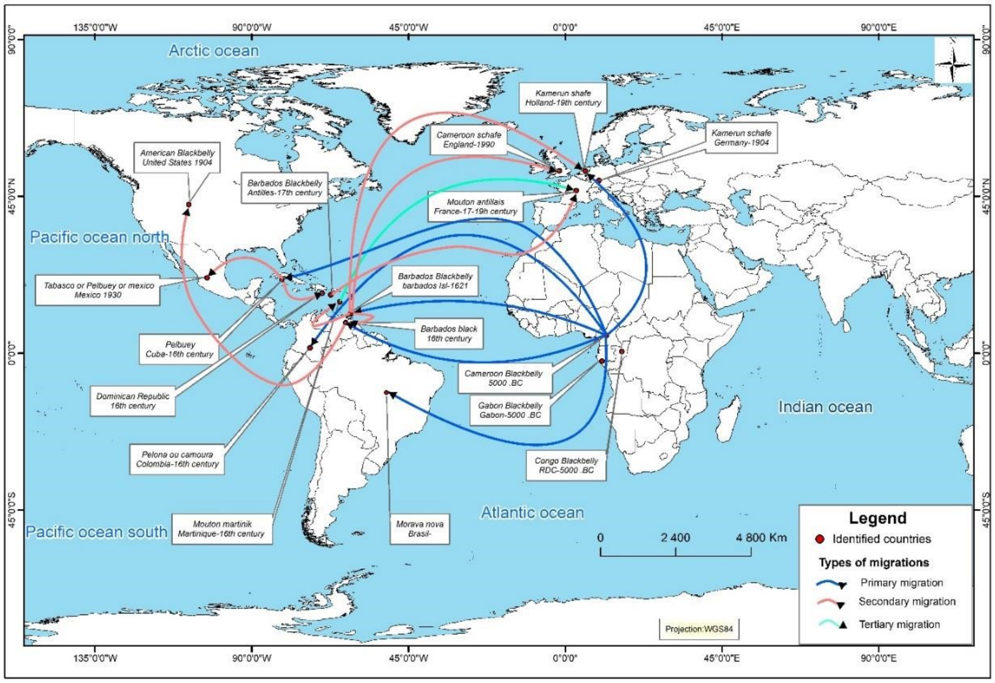

Figure 6: Map of Blackbelly sheep dispersal in the world

Primary migration

The very first documented migrations of the Blackbelly from Central Africa (its cradle) to other horizons which dated back from the 15th century. This migration was then factored by two main phenomena: the slave trade and the colonization. If the mechanisms are not clearly established on the primary migration of Blackbelly, it is recognized by several authors (Fitzhugh and Bradford, 1980; Mason, 1980; Shelton et al., 1990) that, these two phenomena had a great impact on the spread of this genetic material. The main countries with this primary migration waves were Barbados, Cuba, Brazil, Colombia and Trinidad and Tobago.

Secondary migration

After the transfer of this genetic resource from Africa to the Caribbean countries, other countries interested in the qualities of production, adaptation but especially disease resistance imported the Blackbelly sheep mainly from the Barbarian islands. These were mainly the United States of America and Martinique, and surprisingly from these sites to Europe (Great Britain and the Netherlands). In these new destinations, new varieties of Blackbelly sheep were developed with emphasis either on crossing or later on selection. The main mechanisms were importation and colonization (Rastogi et al., 1980, Naves et al., 2011).

Tertiary migration

The migration of Blackbelly sheep is continuous, within the various the host countries for several purposes, mainly adaptation. In the most recent migration traceable activities, France and Martinique were involved; it resulted in the development of the *Martinik breed* (Mathieu, 1997). Transboundary migration is also very common in Central Africa, though less documented.

Conclusion

The *Blackbelly sheep*, originated in tropical humid Africa, probably Central Africa and has become very popular throughout years. The breed popularity is based on its ability to adapt and its high prolificacy. Blackbelly sheep has been widely distributed throughout the world through the slave trade and triangular trade. There are various varieties, but all indicating the tendency for meat. In order to enhance its genetic potential, several countries have developed more productive genetic types through selection and double-crossing. The original traits remained the same in many of these breeds thus favoring the identification of their origin as *Cameroon Blackbelly sheep*. These movements of the colonial era will have had a major impact in the dissemination of animal material between Europe, Africa and America. Deeper molecular investigations are to be done to attest the genetic distances and news traits, while Cameroon government should invest more for the protection, ownership and valorization of this important biodiversity resource.

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