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#### Research article

# Snake species assemblages across habitat types in four departments of the Republic of Congo, with emphasis on medically-relevant venomous species

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#### ABSTRACT

Snakebite is a neglected public health crisis in sub-Saharan Africa. There is a particular lack of data (on snakes and snakebite) from the central African region. This study was conducted in the departments of Likouala, Sangha, Cuvette-Ouest and Kouilou, in the Republic of Congo. The objective was to inventory snakes in the four localities with particular emphasis on medically relevant venomous snakes in order to improve knowledge of snakes in order to minimize the risks of snakebite envenomation to humans. Two methods (active and passive) were used to collect specimens from different habitats. Fifty-one (51)snake specimens including 14 medically relevant snake specimens representing 3 families, 3 subfamilies, 5 genera, and 6 species, in addition to 22 harmless species, were collected. We found a high number of medically important venomous species in Okoyo, Mokéko and Mvouti districts with 3 species each. The highest number of medically important venomous species was recorded in natural forests and human habitations, five and four species respectively. The species obtained (Atheris squamigera, Bitis arietans, Bitis gabonica, Dendroaspis iamesoni, Naja melanoleuca and Toxicodryas blandingii) are medically relevant toxic species according to the WHO classification. Further studies would be necessary to assess the epidemiology of bite risks snakes and educate the public to minimize accidental human-snake contact.

# 1. Introduction

Snakes are present in many parts of the world and conflict between humans and snakes can be a threat to public health, particularly in rural tropical areas [1]. Out of more than of approximately 4000 species of snakes globally, fewer than 700 species can potentially cause dangerous envenomations to humans [2]. For clarity, we will refer to species capable of causing such envenomations in humans as "medically-relevant venomous snakes" as opposed to "harmless" snakes, rather than "venomous" versus "non-venomous". The reason for making this distinction is that many snake species are venomous in the sense that their venom helps them subdue or digest

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their prey, but are not capable of causing envenomations that are dangerous to humans. The World Health Organization classifies medically important venomous snakes into categories as follows [3]:

Category 1: Snakes that bite frequently and are associated with serious or life-threatening envenoming.

Category 2: Snakes that bite frequently, but rarely cause serious or life-threatening envenoming.

Category 3: Snakes that bite rarely, but are capable of causing severe or life-threatening envenoming.

Category 4: Snakes that bite rarely, and have not caused significant envenoming.

Category 5: Other potentially venomous snakes which have not caused documented bites.

Republic of Congo is an equatorial country on the northwest bank of the Congo river in central Africa with a population of 5,125,821 and per capita GDP of \$2,444. Congo's ecoregions include tropical forests and savannah and a climate characterized by heavy precipitation, high temperatures and high humidity. The true burden of snakebite in central Africa remains largely unknown due to an absence of relevant public health data [4], but an abundance and diversity of dangerous snakes combined with rural populations, minimal infrastructure, and reliance on high-risk activities (e.g. agricultural labor and pastoralism) suggest that the impact is substantial [5].

The abundance and distribution of snake species varies depending on diversity of natural environments and the specificity of the ecological requirements specific to each species [6]. Our goal with this study was to inventory snake species in four different localities in the Republic of Congo, with particular emphasis on medically relevant venomous snakes in order to improve knowledge of snakes to minimize the risks of snakebite envenomation for humans.

#### 2. Methods

# 2.1. Study sites

The Republic of Congo is divided administratively into 12 departments, with each department subdivided into districts. This study was carried out in 4 of these departments (Likouala, Sangha, Cuvette-Ouest, and Kouilou). This study was conducted in 4 districts belonging to the departments of Likouala (Enyelle), Sangha (Mokéko), Cuvette-Ouest (Okoyo) and Kouilou (Mvouti) (Fig. 1). We worked in 4 localities of each district and used abbreviations for each locality: In Mokéko and Enyellé, we worked in the villages bearing the name of the district (MKO and ENL). In the district of Okoyo we worked in the locality of Lékety (LKT) and in the district of Mvouti, we worked in the biosphere reserve of Dimonika (RDB).

# 2.2. Description of sampling sites and habitats in the study area

The study took place in the districts of Okoyo ( $1^{\circ}27'41.50"S$  and  $15^{\circ}04'34.32"E$ ), Enyellé ( $2^{\circ}49'03.03"N$  and  $18^{\circ}01'06.15"E$ ), Mokéko ( $1^{\circ}33'45.36"N$  and  $15^{\circ}56'36.78"E$ ) and Mvouti ( $4^{\circ}22'36.39"S$  and  $12^{\circ}20'46.99"E$ ). The district of Okoyo ( $1^{\circ}27'41.50"S$  and  $15^{\circ}04'34.32"E$ ) is located in the Cuvette-Ouest department in the northwestern part of Congo. The climate is of the forest Guinean or lower Congolese type characterized by a high temperature with a low amplitude variation, a rainfall varying between 1,400 mm and 2,200 mm, a dry season of 2–4 months (between June and September), particularly high atmospheric humidity (70-90%) and a daily amplitude that exceeds 40% [36].

The district of Enyellé ( $2^{\circ}49'03.03"$ N and  $18^{\circ}01'06.15"$ E) is located in the department of Likouala. The climate in this department is similar to equatorial and humid tropical climates of the Guinean forest type. This climate is characterized by precipitation of 1,600 to 1,800 mm with an interannual variability of 10-15 %; a dry season of 40 days from December to January; an intra-pluvial decline in July; an annual average temperature of 25-26 °C with an amplitude of  $1-2^{\circ}$  and diurnal from 9 to 140; a relative air humidity of 84-86 % all year round [37].

The district of Mokeko (1°33'45.36"N and 15°56'36.78"E), is located in the department of Sangha. Located in the North West part of Congo. The climate of the Sangha is of the equatorial type, characterized by a 4-season rainfall distribution, low temperature variations during the year, high atmospheric humidity and good insolation. The annual rainfall is between 1500 and 1700 mm, with 2 minimums and 2 maximums [38].

The district of Mvouti (4°22'36.39"S and 12°20'46.99"E) is located in the department of Kouilou which presents two large units which can be distinguished on the basis of the relief and the vegetation: The maritime facade or coastal plain and Mayombe [39]. The district of Mvouti is located in the heart of Mayombe which is a mountain range parallel to the Atlantic coast, very rugged but low altitude, whose highest point is Mount Foungouti (930 m). The climate of Mayombe is of the lower Congolese subequatorial type. This climate is characterized by average annual rainfall, around 1700 mm, spread over 8 months, from October to May. During the dry season cloudiness is at its maximum, with daily fogs, and the relative humidity remains very high all year round.

Localities and sampling sites were chosen according to their accessibility, type and degree of vegetation cover. This made it possible to define five types of habitats: natural forests (FONA), Small crops (SCR), Savannah (SAV), human habitations (HH) and wetlands (WE).

We subdivided the habitat types into eight micro-habitats as follows: Soil (SO), leaf litter (LI), trees (Tr), grass (Gr), dead tree trunks (Tt), manioc patches (Pl), water (Wa) and Stone (St).

# 2.3. Sampling and identification methods

Survey personnel carried out sampling in Dimonika Reserve biosphere at Mvouti between September 20 and October 10, 2020. We

sampled in Lékety from November 6 to 28, 2020. We sampled in Enyellé between February 14 and March 6, 2020 and Mokéko between July 29 and August 17, 2021. Five stations were selected in each locality in which we placed a line of traps for a total of 20 stations representing the different habitats and microhabitats in the study area (Fig. 2). Sampling was carried out using active and passive (or trapping) methods, following the methodologies of [10,40,41]. A GPS point was recorded with each sighting or capture of a snake. Collecting was carried out every day between 9 a.m. and 5 p.m. during the day of the collection periods. The sampling period was 3 weeks of collecting in each locality. Active searches were done randomly in the different sampling sites in the study area because our movements and those of the guides did not follow a transect, we used the sight hunting technique described by Ref. [15]. Active searches are essentially based on systematic searching in places likely to harbor snakes. These searches were sometimes done along tracks and/or paths already existing in forests (natural and planted) and savannah, and near human habitations or wetlands. Searching of microhabitats involved turning over piles of debris on the ground, dead leaves, grasses, inspecting tree stumps, bark, dead tree trunks, bricks and/or pebbles. Trapping involves either placing fishing nets in a watercourse to capture aquatic species or setting pitfall traps using plastic buckets buried in the ground. Two types of pitfall traps were used in this study. The method consisted of installing lines of pitfall traps made up of 4 plastic buckets and three strips of transparent or black plastic sheeting, 10 m each, forming a drift fence. During this study, lines of traps were placed parallel to the bed of streams or swampy areas. For each trap line, plastic buckets were placed at each end and partway along the drift fence. Each bucket, buried flush with ground level at a height of 29 cm, a circumference at the top of 91 cm at the base of 71 cm. The bottom is perforated with small holes of 5 mm in diameter to allow the evacuation of rainwater. These holes are covered with a fine mesh to prevent small specimens from escaping. The barrier is vertical and 50 cm high, it is supported by wooden stakes. The plastic sheeting of the fence forms a horizontal fold at ground level, which is covered with soil and litter to prevent specimens from passing underneath. In the middle of each section of the drift fence, a funnel trap is placed 5 m between two buckets. These (funnel traps) are covered with leaf litter to create an artificial habitat for the snakes. The traps are inspected once a day in the morning. The second pitfall method (with the drift fence arranged like spokes on a wheel rather than in a single line) is that described by Ref. [42] and used by Ref. [43]. Unlike the first, this method has the advantage of taking a large variety of snakes from different directions of the biotope sampled. The trap consists of three diversion barriers, each 10 m long, arranged in a "Y" shape designed using plastic sheeting and fixed to wooden stakes buried in the ground, four plastic buckets of 20 L each, buried flush with ground level and six funnel traps Each of the ends of the three diversion barriers ends in a bucket. These are connected by a bucket installed in the center of the pit trap. Around the central bucket, the rows of traps are arranged so that they drift two by two, an angle of 120°. Halfway to each diversion barrier, a pair of funnel traps, covered with leaf litter, is installed. The traps were examined daily throughout the sampling period. The gill net method can be used to capture aquatic species of snakes [10]. This method consists of placing 2.5 cm gillnets in streams, lakes and all water reservoirs that may contain snakes. At each sampling site, 30 m of 2.5 cm gillnets (i.e. 3 nets of 10 m each) were placed in the water points. Each fishing net was 10 m long and 50 cm high. Supported on both sides by wooden stakes, the fishing nets were placed in favorable and shallow places to facilitate their relief. In addition, 40 cm in height of the net was submerged, 10 cm emerged was left above to catch species that would try to swim on the surface of the water.

The specimens collected were transported to the laboratory, photographed and identified using the available identification keys [13,20–23,44]. Family level classification is consistent with that proposed by Ref. [45]. After identification, the specimens were kept (in collection) in the herpetology laboratory of the National Institute for Research in Exact and Natural Sciences (IRSEN) in Brazzaville.

#### 2.4. Statistical analysis of data

The data collected was subjected to statistical analysis. The species richness, the relative abundance and frequencies were calculated using Microsoft Excel 2007. The diversity indices were calculated using the PAST software (V.3.26) to estimate the richness and diversity of the snake fauna of each locality. Locality and habitat similarity index dendrograms were plotted from the Bray-Curtis association matrix with PAST software (V.3.26).

We used Duellman's Faunal Similarity Index to compare the four localities and the different habitat types. It makes it possible to know whether the faunal groups of the different compared biotopes belong to the same animal community. Duellman's faunal similarity index (K) compares lists of species found at two sites as follows:  $K = 2C \times 100/(A + B)$ 

Where K = the percentage of species common to both groups; A = total number of species in group 1; B = total number of species in group 2; C = total number of species common to both groups. If K = total number of species are considered to represent the same community [46,47].

# 3. Results

#### 3.1. Composition of the snake fauna

A total of 51 individual snakes were sampled in the five habitats of the study area, including 14 medically relevant venomous snakes representing six species. In the habitats, the number of specimens collected was 14 respectively in the natural forest (FONA) and wetlands (WE), 10 near human habitations (HH), seven in savannah (SAV) and 6 in the small crops (SCR) (e.g. cassava plantations) (Table 1). In the micro-habitats, out of the 51 specimens, the majority, 39, were collected on the soil (So), 5 in the grass (Gr), 2 on the branches of trees (Tr) and in the water (Wa). The micro-habitats: leaf litter (LI), dead tree trunk (Tt) and stones (St) each have a single specimen, the manioc patches plantations (PL) micro-biotope had no specimens.

# 3.2. Relative abundance of snakes by family

The relative abundance of individual snakes per family ranged from 39 % to 4 % of total numbers of individuals. Snake species belonging to the Colubridae family were the most abundant in the samples with 39 %, followed by the Lamprophidae family with 27 %

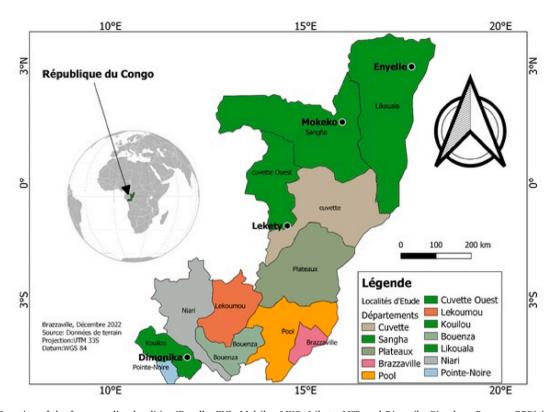


Fig. 1. Location of the four sampling localities (Enyelle: ENL, Mokéko: MKO, Lékety: LKT, and Dimonika Biosphere Reserve: RBD) in four departments (Likouala, Sangha, Cuvette-Ouest, and Kouilou) of the Republic of Congo.



Fig. 2. Location of sampling sites within the four sampling localities.

Table 1
Snake species collected, organized taxonomically (Family, Subfamily, Genus and species), with numbers of individuals collected in each habitat type (FONA: Natural forest; SAV: Savannah; SCR: Small crops; HH: Human habitations; WE: Wetlands) and microhabitat (SO: Soil, LI: Leaf litter; Gr: Grass; Tr: Trees; Tt: Trees trunk; PL: manioc patches; Wa: Water; St: Stone). Medically relevant venomous species are indicated with an \*, and their WHO venomous snake category is listed.

Families	Subfamilies	Genera and WHO Species categ.		Field site			Habitat type				Microhabitat type											
				Species Codes	ENL	RBD	МКО	LKT	FONA	SAV	SCR	НН	WE	So	Li	Gr	Tt	Tr	Wa	Pl	St	Total
TYPHLOPIDAE	Afrotyphlopinae	Afrotyphlops congestus		Afco	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1
		Afrotyphlops sp		Afsp	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1
PYTHONIDAE		Python sebae		Pyse	1	0	0	1	0	1	0	0	1	1	0	0	0	0	1	0	0	2
	Viperinae	*Atheris squamigera	3	Atsq	1	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0	2
		*Bitis arietans	1	Biar	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1
		*Bitis gabonica	3	Biga	0	2	1	0	2	0	0	1	0	3	0	0	0	0	0	0	0	3
LAMPROPHIIDAE	Psammophiinae	Psammophis phillipsii		Psph	0	0	0	3	1	0	1	1	0	2	0	1	0	0	0	0	0	3
		Psammophis silibans		Pssi	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1
	Aparallactinae	Polemon barthii		Poba	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1
	Lamprophiinae	Boaedon lineatus		Boli	0	0	0	2	0	0	2	0	0	2	0	0	0	0	0	0	0	2
		Boaedon olivaceus		Bool	1	2	0	0	0	0	1	1	1	3	0	0	0	0	0	0	0	3
		Bothrophtalmus lineatus		Botli	2	0	0	0	2	0	0	0	0	2	0	0	0	0	0	0	0	2
		Hormonotus modestus		Homo	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1
		Mehelya poensis		Меро	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1
ELAPIDAE	Elapinae	*Dendroaspis jamesoni	1	Deja	1	0	0	2	1	1	0	1	0	2	0	0	0	1	0	0	0	3
		*Naja melanoleuca	3	Name	0	1	2	1	2	0	0	1	1	4	0	0	0	0	0	0	0	4
COLUBRIDAE	Grayiinae	Grayia ornata		Gror	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1
	Colubrinae	Dasypeltis fasciata		Dafa	0	0	1	1	0	0	1	1	0	2	0	0	0	0	0	0	0	2
		Dipsadoboa duchesnii		Didu	0	0	0	2	0	2	0	0	0	2	0	0	0	0	0	0	0	2
		Dipsadoboa unicolor		Diun	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1
		Dipsadoboa viridis		Divi	0	0	2	0	0	0	0	0	2	2	0	0	0	0	0	0	0	2
		Hapsidophrys smaragdina		Hasm	2	1	1	0	1	0	0	2	1	1	1	2	0	0	0	0	0	4
		Philothamnus heterodermus		Phhe	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1
		Philothamnus heterolepidotus		Phle	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1
		Rhamnophis aethiopissa		Rhae	0	2	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0	2
		*Toxicodryas blandingii	5	Tobl	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1
		Toxicodryas pulverulenta		Тори	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1
	Natricinae	Natriciteres fuliginoides		Nafu	0	2	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	2
6 families	9 subfamilies	20 genera 28 spec	ies		11	18	6	16	14	7	6	10	14	39	1	5	1	2	2	0	1	51

and the Viperidae and Elapidae families with 12 % and 14 % respectively. The Typhlopidae and Pythonidae families are the least represented with 4 % (Fig. 3).

# 3.3. Species richness of snakes by habitat

The largest number of snake species was inventoried in natural forests (FONA) and wetlands (WE), respectively 11 species (27 %) and 12 species (27 %) with the same number of individuals; human habitations (HH) with 9 species or 20 %; savannah (SAV) with 6 species, representing 14 % of the samples and small crops (SCR) with 5 species, equal to 12 % of the samples. (Fig. 4).

#### 3.4. Species richness of snakes by micro-habitat within habitat types

Snakes are unevenly distributed in the habitats of the study area. The percentage of snakes collected on the ground as opposed to other microhabitats in each habitat varied as follows: Ninety percent (90 %) of snakes were collected on the ground in the Human habitation (HH), 88 % in savannah (SAV), 77 % in natural forest (FONA), 67 % in small crop habitat (SCR), and 57 % in wetlands (WE). In the other microhabitats making up each habitat type, the specific contribution varied from 7 to 17 % contributing to the specific richness of the environments surveyed (SCR, SAV, WE, and HH). 17 % of species were found in grasses and 17 % in trees in small crop habitats (SCR). In the human habitation (HH) habitat type, 10 % were collected on grasses while 14 % were collected on grasses in the wetland (WE) habitat type. No snakes were collected in the manioc patches (Pl) microhabitat of any habitat type (Fig. 5).

# 3.5. Species richness of snakes by micro-habitat

Table 2 shows that the snake fauna was much more active on the ground where 28 snake species (representing 20 genera and 6 families) were captured and/or observed. This is followed respectively by grass with 4 species (4 genera and 2 families), trees and water, each with 2 species (divided into 2 genera and 2 families) and dead tree trunks and stone with 1 species. No species was captured in the micro-habitats cassava plantations (Pl).

# 3.6. Index of diversity and richness of snakes by habitat types

According to the indices of Shannon and Simpson, the species diversity of WE (H' = 2.44; 1-D = 0.90) was higher than in FONA (H' = 2.34; 1-D = 0.89) and HH (H' = 2.16; 1-D = 0.88). The lowest diversity was recorded at SCR (H' = 1.56; 1-D = 0.5) and at SAV (H' = 1.71; 1-D = 0.81). The evenness index (J) of the five habitats is less than 1, which suggests a significant variation in the numerical density of the species present in the sampling sites (Table 3).

#### 3.7. Similarity between biotopes

The structure of snake's communities of the habitat types was compared by a hierarchical classification. The dendrogram of similarity between biotopes shows four clusters, the first formed by the cluster composed by HH-FONA. The second group formed by SCR and cluster HH-FONA. The third group is formed by WE which is associated the first two groups and the fourth group is formed by the SAV and the 3 first clusters (Fig. 6).

# 3.8. Similarity between localities

The structure of snake's communities of the different localities was compared by a hierarchical classification. The dendrogram of similarity between localities shows three clusters, the first is formed by cluster composed by MKO-RDB. The second group is formed by ENL and the cluster MKO-RDB. The third group is consisted LTK and the first two groups (Fig. 7).

#### 3.9. Duellman K Faunal similarity index (%) localities

The faunal similarity index of the snake species between the four localities studied varied between 0 and 25 % (Table 4). These indices of faunal similarity all fall below 50 %, indicating a difference between the two faunas being compared. The two most similar faunas were Dimonika Biosphere Reserve (RDB) and Mokéko (MKO), with a similarity index (K) of. 25 %. The next most similar were Enyellé (ENL) and Dimonika Biosphere Reserve (RDB) (K = 20.69 %), Mokéko (MKO) and Lekety (LKT) (K = 18.18 %), Enyellé (ENL) and Mokéko (MKO) (K = 11.76 %). The least similar were Enyellé (ENL) and Lekety (LKT), (K = 7.41 %), and Dimonika Biosphere Reserve (RDB) and Lekety (LKT), (K = 5.88 %).

# 3.10. Duellman K Faunal similarity index of habitats

The faunal similarity index of the snake species between the habitat type varied between 0 and 42.86% (Table 5). The habitat types with the most similar snake faunas were small crops (SCR) and human dwellings (HH), (K = 42.86%). The next most similar were natural forest (FONA) and human dwellings (HH), (K = 40%); wetlands (WE) and human dwellings (HH) (K = 28.57%); wetlands (WE) and natural forest (FONA) (K = 26.09%). The least similar, with K values below 20% were natural forest (FONA) and small crops

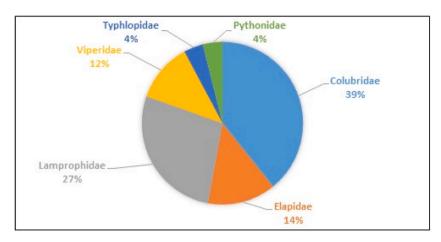


Fig. 3. Relative abundance of total number of individual snakes collected, by family.

(SCR) (K = 12.50 %); savannah (SAV) and natural forest (FONA) (K = 11.76 %); savannah (SAV) and human habitation (HH) (K = 13.33 %), savannah (SAV) and wetlands (WE) (K = 11.11 %); (WE) and small crops (SCR) (K = 11.76 %); and savannah (SAV) and small crops (SCR) (K = 0).

In order to better understand the importance of medically relevant venomous snakes in the surveyed localities, their abundance and their diversity were calculated.

# 3.11. Relative abundance of medically-relevant venomous snakes by family

Harmless species represent 72.5 % of the samples (37 individual snakes), representing 22 species. The 14 medically relevant venomous snakes specimens represent three families, three subfamilies, five genera and six species. The Elapidae family accounts for most of the medically-relevant venomous snakes with 7 individuals (50 %), followed by the Viperidae family with 6 individuals (43 %) and the Colubridae family with 1 individuals (7 %) (Fig. 8).

# 3.12. Species richness of medically-relevant venomous snakes by locality

The analysis of Fig. 9 shows that we collected the most medically-relevant venomous snakes species (3 species) in the localities of Lékety, Mokéko (MKO) and Dimonika (RBD). In the locality of Enyellé (ENL) we identified two medically-relevant venomous snakes species (Fig. 9).

# 3.13. Species richness of medically-relevant venomous snakes by habitat

The greatest number of medically-relevant venomous snakes species was inventoried in natural forests (FONA) and human habitations (HH), i.e. respectively 5 species with 7 individuals (50 %) and 3 species (21.43 %); monitoring of the savannah (SAV) with 2 species, 2 individuals or 14.29 %; in the wetland habitats (We) and Small crops (SCR) one species with 1 individual each, i.e. 7.14 % was collected (Fig. 10).

# 3.14. Distribution map of medically relevant venomous snakes

Fig. 11 shows the distribution of venomous species captured in the different localities of the study area.

#### 3.15. Description of medically-relevant venomous snakes species obtained during the study (Fig. 12)

#### 3.15.1. Colubridae: colubrinae

*Toxicodryas blandingii* (Hallowell, 1844): A single specimen was captured while actively searching on a tree trunk in the forest near Pako village in Mokéko district. This large snake with nocturnal habits is strictly arboreal in dense primary and secondary forests, in gallery forests and even in humid wooded savannahs (Fig. 12a).

# 3.15.2. Elapidae: Elapinae

**Dendroaspis jamesoni** (Traill, 1843): Three specimens were captured respectively 2 specimens at Lekety and 1 specimen from Enyellé specimen (Fig. 12b). This strictly diurnal, very fast species is semi-arboreal. It lives in open primary and secondary forests, in gallery forests and humid savannahs. It sometimes ventures into crop plantations and gardens, and sometimes even into towns, especially during the flowering period of fruit trees. Their bite is extremely dangerous for humans. Not very aggressive despite its bad

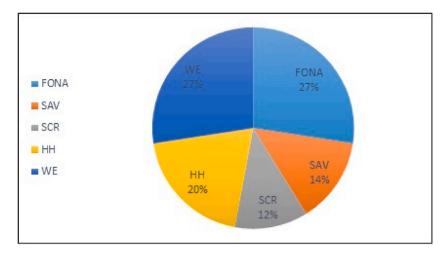


Fig. 4. Relative abundance of total number of snake species collected, by habitat type (FONA: Natural forest; SAV: Savannah; SCR: Small crops; HH: Human habitations; WE: Wetlands).

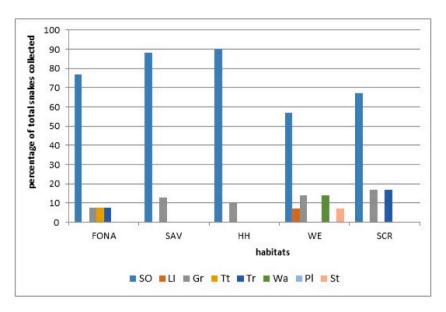


Fig. 5. Relative abundance of snakes collected, by micro-habitat within each type of habitat (FONA: Natural forest; SAV: Savannah; SCR: Small crops; HH: Human habitations; WE: Wetlands. SO: Soil, LI: Leaf litter; Gr: Grass; Tr: Trees; Tt: Trees trunk; PL: manioc patches; Wa: Water; St: Stone).

Table 2
Number of individual snakes and number of families, genera and species of snakes collected in each micro-habitat (SO: Soil, LI: Leaf litter; Gr: Grass; AR: Trees; Tt: Trees trunk; Pl: Plantations; Wa: Water; St: stone).

Microhabitat	Number of individual	Number of families	Number of genera	Number of species
SO	39	6	20	28
LI	1	1	1	1
Gr	5	2	4	4
Tt	1	1	1	1
Tr	2	2	2	2
Wa	2	2	2	2
PL	0	0	0	0
St	1	1	1	1

Table 3 Indices of snake species diversity and richness by habitat types (FONA: Natural forest; SAV: Savannah; SCR: Small crops; HH: Human habitations; WE: Wetlands).

	FONA	SAV	НН	WE	SCR
Taxa_S	11	6	9	12	5
Individuals	14	7	10	14	6
Simpson_1-D	0.898	0.8163	0.88	0.9082	0.7778
Shannon_H	2.342	1.748	2.164	2.441	1.561
Equitability_E	0.9767	0.9755	0.9849	0.9823	0.9697

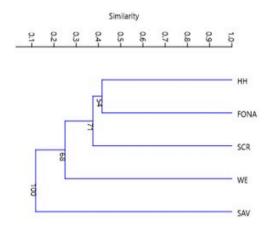


Fig. 6. Dendrogram of similarity indices between the five habitat types (FONA: Natural forest; SAV: Savannah; SCR: Small crops; HH: Human habitations; WE: Wetlands).

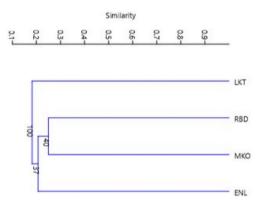


Fig. 7. Dendrogram of similarity indices between the four localities (Enyellé: ENL, Mokéko: MKO, Lékety: LKT, and Dimonika Biosphere Reserve: RBD).

reputation, accidents are rare.

*Naja melanoleuca* Hallowell, 1857: Four specimens were collected during this study 2 in Mokéko, 1 in Dimonika and 1 in Lekety (Fig. 12c). The forest cobra is a terrestrial and ubiquitous species with nocturnal and diurnal habits. It is found on the ground in all environments, starting from the dense primary forest, the gallery forests to the savannahs. It also frequents wetlands, crop plantations and towns where it is happy to be found around or in human dwellings. It is hidden during the day and it comes out at night.

# 3.15.3. Viperidae: Viperinae

Atheris squamigera Hallowell, 1856: Two specimens were sampled, on the ground at Dimonika and on a shrub at Enyellé (Fig. 12d). This strictly arboreal species is often observed on the branches of tall trees.

*Bitis arietans* Merrem, 1820: A single specimen was sampled, on the ground at Lekety, Okoyo district (Fig. 12e). This viper is very dangerous because of its very active venom on muscle tissue, and is responsible for several accidents in sub-Saharan Africa.

*Bitis gabonica* Duméril, Bibron, & Duméril, 1854: the three specimens were captured on the ground, one at Mokéko in the clear forest, and two at Dimonika respectively near human habitations and in the forest (Fig. 12f).

Table 4
Duellman K Faunal Similarity Index (%) comparison of localities (ENL: Enyellé; RBD: Dimonika Biosphere Reserve, MKO: Mokéko; LKT: Lékety).

Localities	ENL	RBD	МКО	LKT
ENL	0	20.69	11.76	7.41
RBD		0	25	5.88
MKO			0	18.18
LKT				0

Table 5

Duellman K Faunal Similarity Index (%) comparison of habitat types (FONA: Natural forest; SAV: Savannah; SCR: Small crops; HH: Human habitations; WE: Wetlands).

Biotopes	FONA	SAV	НН	WE	SCR
FONA	0	11.76	40	26.09	12.50
SAV		0	13.33	11.11	0
HH			0	28.57	42.86
WE				0	11.76
SCR					0

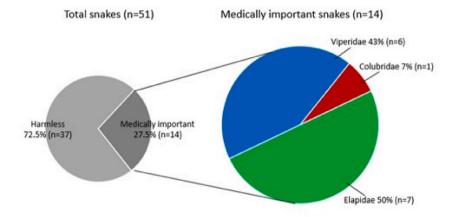


Fig. 8. Abundance of medically-relevant venomous by family.

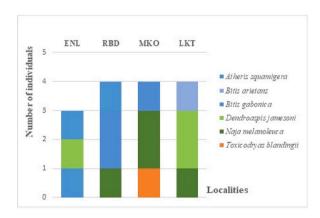


Fig. 9. Species richness of medically-relevant venomous snakes by locality (ENL: Enyellé; RBD: Dimonika Biosphere Reserve, MKO: Mokeko; LKT: Lékety).

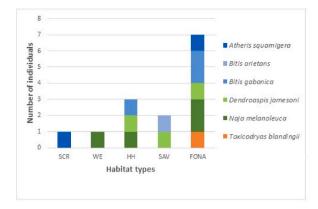


Fig. 10. Species richness of medically-relevant venomous snakes by habitat (FONA: Natural forest; SAV: Savannah; SCR: Small crops; HH: Human habitations; We: Wetlands).

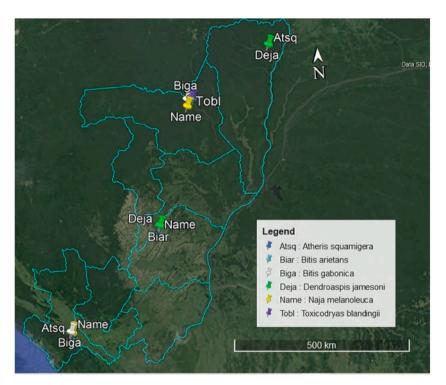


Fig. 11. Distribution of medically-relevant venomous snakes species in the four districts.

#### 4. Discussion

This study aimed to inventory snakes in four localities with a greater emphasis on medically-relevant venomous snakes. The samples of snakes listed in the four localities is dominated by species of the Colubridae and Lamprophiidae families. These two families are followed by Viperidae then by that of Elapidae and Typhlopidae. These results are similar to those obtained by Ref. [7] in Brazzaville and by Ref. [8] in the Kouilou Basin who also observed the dominance of Colubridae. For these authors, the family of Colubridae is followed by that of Elapidae and Viperidae [9]. in Kouilou and [10] in Likouala observed the same trend marked by the predominance of Colubridae. This family is followed in both cases by that of the Viperidae. Furthermore [11], also noted the dominance of the Colubridae family in the Sangha followed by that of the Elapidae. The strong representation of Colubridae is explained by this family being the largestin numbers of species and genera. This observation corroborates that made by Ref. [12]. The large numbers of Lamprophiidae relative to earlier studies represent taxonomic revision of snake families in recent decades (reviewed by Ref. [13]). The Lamprophiidae family includes as subfamilies that were once families unto themselves (Atractaspinae for example) and subfamilies that were previously grouped among the Colubridae (e.g. Aparallactinae, Lamprophiinae, Psammophiinae and Prosymninae). The specific richness and the Shannon index indicate that the population of snakes is more diver-sified in WE (H' = 2.44), FONA (H' =



Fig. 12. Representative images of the six medically-relevant venomous snake species collected: (a) *Toxicodryas blandingii* (Blanding's Tree Snake); (b) *Dendroaspis jamesoni* (Jameson's mamba); (c) *Naja melanoleuca* (Forest cobra); (d) *Atheris squamigera* (Rough-scaled bush viper); (e) *Bitis arietans* (Puff adder); (f) *Bitis gabonica* (Gaboon viper). (Photographed by LBM).

2.34) and HH (H' = 2.16) than in SCR (H' = 1.56) and SAV (H' = 1.71). The surveyed habitats are noted by an equitability around 1 suggesting that the population of snakes does not undergo large variations in their distribution in the different habitats.

The results of this study show found medically-relevant venomous snakes species to be most numerous in forests and near human habitations. These results are similar to those obtained in Cameroon [6], and Ivory Cost [14], where forest habitat was found to have a higher percentage of venomous species than other habitat types. According to these authors, these results could be explained by the fact that the forest, with its vegetation cover and dense undergrowth, offers more habitat complexity and niches for wildlife. On the other hand, the observation of venomous species around human habitations would be due to the presence of roosts and the availability of food resources (small rodents, amphibians, chicken eggs, piles of household waste, etc.) and by the fact that certain snakes adapt to the modification of their habitats by coming into contact with humans [14,15]. According to Ref. [16] species obtained near dwellings could be considered as anthropophilic species whose demography it is important to follow in order to minimize accidental human-snake contacts.

The results show that the biotope impacts both the number and the type of species observed. Indeed, in wetlands and small crops, although the species are different, their number is identical in the two biotopes. This difference would be due to the trophic and habitat requirements of each species. The three sampled snake families were abundantly represented in the micro-habitats: soil, trees and dead tree trunks. The results show the same trends in the other micro-habitats (grass, leaf litter, pools of water, trees and dead tree trunks). These results are lower (in terms of numbers of families represented in the our sample) than those obtained in several studies with

figures varying between four and six families [7,11,17], and other who obtained seven families in all the microbiotopes combined [8–10,18].

The similarity of the population of snakes of all the habitats and four localities taken two by two did not exceed 50 %. This means that the populations of snakes from these 4 localities and 5 bio-topes present a weak affinity if not no affinity between the species of these different environments. It is therefore obvious that these stands are clearly individualized and therefore belong to different animal communities. This observation corroborates that of [17] in the Pool department in Congo and differs from that of [19] in the forest reserve of Takamanda and its surroundings in the south-west of Cameroon.

In this study, two species of the genus *Toxicodryas* were collected (*Toxicodryas blandingii* & *Toxicodryas pulverulenta*). Many authors have shown that these two species are arboreal and nocturnal. They live in dense primary forests, gallery forests and savannas [20,21]. However, it is not uncommon to find them in the gardens of urban agglomerations [9,22]). *Toxicodryas pulverulenta* has been reported in Congo in the following localities: Dimonika, Makoua, Sibiti, Ouesso, Liouesso and Brazzaville [8,18,23]. While *Toxicodryas blandingii*, has been reported in Brazzaville, Dimonika, Ngangalingolo, Ouesso, Bassin du Kouilou, Meya. It has also been reported in Ngabé and Zanaga [17,24].

Dendroaspis jamesoni is restricted to forest areas [23] and sometimes ventures into wet savannahs, food plantations and gardens, and even into built-up areas [22]. This author adds that this strictly diurnal and semi-arboreal species does not disdain moving on the ground and can even be relatively clumsy when perched. However [22], showed that this species occupies the same ecological niche as *D. viridis* in West Africa. The species *D. jamesoni* is also present in western Togo [25]. In Congo, the presence of this species has been mentioned by authors such as: [9] in Mayombe, in degraded areas near Pointe-Noire [26]. This species is also known from Liouesso, Ouesso, Kinkembo, Nganchou down Alima, Brazzaville, Dimonika, Tchissanga, Makoua, Mbomo, Bas-Kouilou, Bouenza and Lékoumou [8,18,24,26,27].

The genus Naja is represented in Congo by 4 species [27]. In this study, only *Naja melanoleuca* was collected both in the forest, at the edge of watercourses and near dwellings. According to Refs. [23,27] *N. melanoleuca* is a species restricted to forest areas where it is always abundant and can also be observed in the savannah near large rivers and dwellings. The presence of this species in Congo has been reported in several localities: Pointe-Noire, Bomassa, Impongui, Bena Dimonika, Makoua, Lekéti, Mbila, Ouesso, Sibiti, Brazzaville and in Lake Titina in Menengué [7–11,18,24,26–28].

The Green Leaf Viper (*Atheris squamigera*) was found on the forest floor and on a shrub in a field. This species can be found on leaf litter or on rocks in primary and secondary forests and in tree plantations [20–23]. The presence of this species in Congo has been reported from Ile Mbamou, Liouesso, Ouesso, Kebara, Odzala, Alima-Leketi, Sibiti, Simombondo, Tchissanga, Menengué, Djoumouna, Kinkembo and Brazzaville [8.17,18,23].

In this study, 2 species of the *Bitis* genus were collected (*Bitis arietans* and *Bitis gabonica*). *Bitis arietans* is a strictly savannah species. The presence of this species has been reported in Odzala, Impongui, Kinkembo, Mayoumina and Brazzaville [7,10,18], Ewo, Odziba, Ngangalingolo, Makoua [23]. *Bitis gabonica* is a ubiquitous species but it is much more reported in forest environments [22]. According to several authors, it is frequent in anthropic environments, food and commercial plantations, including mechanized industrial plantations. It is not exceptional to meet her in town [6]. It has been listed in Dimonika, Mbomo, Impongui, Mossendjo, Ouesso, Mbomo, Bambama and Brazzaville [7,9,11,18,29].

All venomous snakes sampled during this study are medically important as defined by WHO [3]. Thus, a) species of the genus *Toxicodryas* are classified in category 5. Envenomation is characterized by a sudden cardiovascular collapse. The strong proteolytic activity of the venom does not seem to act on blood coagulation and no bleeding disorders are observed ([30] cited by Refs. [20,22]). Its neurotoxic venom is dangerous for humans, with a marked post-synaptic tropism. A case of a non-serious bite from *Toxicodryas blandingii* was reported in Switzerland [31].

- b) The genus *Dendroaspis* is represented by one species in Congo. *Dendroaspis jamesoni* is classified in category 1. The venom is neurotropic and is particularly toxic [22]. During *Dendroaspis* bites, lacrimation, hypersialorrhoea, sweating and diarrhea, miosis, accommodation disorders, photophobia, bronchospasm, vomiting and tremor can be observed in the half hour preceding the cobraic syndrome [32].
- c) *Naja melanoleuca* is classified in category 3 [22]. reveals the neurotoxic nature of its venom. Envenomation causes paralysis of the respiratory muscles leading to death by asphyxiation. Although the other species listed in the Congo have not been collected, it can be noted that *Naja nigricollis* is classified in category 1 while *Naja annulata* and *N. christyi* are classified in category 5 [3].
- d) The species *Atheris squamigera* is classified in category 3. The venom of the viper *Atheris squamigera* is cytotoxic and haemotoxic. During a bite, we notice: nausea, vomiting, diarrhea, consequent pain at the place of the bite, local or even extensive edema (if the bite was made on the fingers, the edema may extend up to the shoulder), local hemorrhage, appearance of fatigue and drowsiness, blisters and local but fairly significant necrosis. The venom has a thrombin-like activity and a strong capacity for platelet aggregation contained in two distinct fractions. The bite of a juvenile individual is dangerous and potentially fatal [33].
- e) Bitis arietans is classified as category 1 while Bitis gabonica is classified as category 3 [3]. The venom of Bitis arietans is haemorrhagic but above all strongly necrotizing [34]. On the other hand, that of Bitis gabonica is highly hemorrhagic and inflammatory [22].

One limitation of the World Health Organisation's system for categorising medically important venomous snakes [3] is the extent to which it depends on record-keeping and documentation of snake envenomations. This means that species whose distribution is limited to regions where documentation is sparse or absent, such as central Africa One limitation of the World Health Organisation's system for categorising medically important venomous snakes [3] is the extent to which it depends on record-keeping and documentation of snake envenomations. This means that species whose distribution is limited to regions where documentation is sparse or absent, such

as central Africa [4], may be overlooked or underestimated. This is particularly true of categories 3 and 5 where it may be hard to distinguish between species which bite rarely versus species whose bites have not been documented. For example, in central Africa, the Forest Cobra (*Naja melanoleuca* or *N. subfulva*) is often encountered in the same habitat as the Water cobra (*N. annulata*), such as waterways in tropical forest where both are often caught in gill nets set by fishermen [10]. The WHO [3] lists the forest cobra, *N. melanoleuca* sensu lato (but see recent taxonomic changes [35]) which is broadly distributed in sub-Saharan Africa, in category 3. *Naja annulata*, on the other hand, whose distribution is limited to central Africa, is listed in category 5 along with opisthoglyph species from genera such as *Toxicodryas* and *Psammophis*.

#### Limitations of the study

The results of this study underestimate the real specific richness of the snake fauna of the study area for three main reasons: the brevity of the actual stays in the field which do not did not make it possible to exploit the various climatic periods; almost exclusively diurnal research due to the danger of night outings, the inaccessibility of certain environments in the sampled sites.

# CRediT authorship contribution statement

Lise Bethy Mavoungou: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Kate Jackson: Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. Joseph Goma-Tchimbakala: Writing – review & editing, Validation, Supervision, Software, Conceptualization.

#### **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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