First Assessment of Nocturnal Lepidopteran Communities in the Forest Savannah Mosaic of the Plateaux Batéké, Southeastern Gabon

Hugues Bikang Bi Ateme, Stephan Ntie, Thibaud Decaëns, and Rodolphe Rougerie

ABSTRACT

Lepidopterans are an important component of central African biodiversity. Indeed, they play an important role as plant pollinators, food source, bio-indicators, and even pests for local crops. However, almost nothing is known about these moths in central Africa, while they are being increasingly threatened by significant landscape changes due mainly to infrastructure constructions and climate change. So, the mosaic of forests and savannah in the Plateaux Batéké (southeastern Gabon) constitutes a unique opportunity to study how Lepidopteran communities might evolve along an ecological gradient with upcoming changes in forest cover in the region. A total of 2824 specimens, representing 14 distinct families or subfamilies, were sampled using light-trapping and sorted into morphospecies, while a subset of 95 samples was further investigated using both morphological and molecular methods. Community comparison of nocturnal Lepidopteran showed that there was a significant difference in terms of abundance only between sampling sessions. Indeed, this could be due to food availability between sampling sessions. In May and June in Gabon, most plants have flowers and fruits and moth families such as Geometridae and Sphingidae emerge at that time. The most represented family in our sampled specimens was the Geometridae, which prefers habitats with permanent river courses as is the case in our sampling area. However, a more comprehensive study using various trapping methods, during several seasonal cycles and with more replicates in each habitat type is needed for a better understanding of the community structure and ecological traits that characterize nocturnal Lepidopteran in the Plateaux Batéké of Gabon.

Keywords: Gabon, Lepidoptera, molecular identification, morphological identification, Plateaux Batéké.

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H. Bikang Bi Ateme

Cellule Scientifique, Agence Nationale des Parcs Nationaux (ANPN), BP 20379, Libreville, Gabon.

(email: huguesbikang@gmail.com)

S. Ntie*

Laboratoire de Biologie Moléculaire et Cellulaire (LABMC), Département de Biologie, Université des Sciences et Techniques de Masuku, Franceville, Gabon.

(email: stephanntie@yahoo.fr)

T. Decaëns

Centre d'Ecologie Fonctionnelle et Evolutive, Université de Montpellier, France.

(email: thibaud.decaens@cefe.cnrs.fr)

R. Rougerie

Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France.

(email: rodolphe.rougerie@mnhn.fr)

**Corresponding Author*

I. INTRODUCTION

Representing more than 80% of all animals, insects are found in all habitat types where they play important roles in ecosystem functioning. For instance, insects of the easily identifiable Lepidoptera order are plant pollinators (Aker & Udovic, 1981; Bopp & Gottsberger, 2004; Hendrix *et al.*, 1987), food source for other organisms (Dennis *et al.*, 2009; Koch *et al.*, 2005), and bio-indicators (Beccaloni & Gaston, 1995; Erhardt, 1985; New, 1997; Valtonen *et al.*, 2006). Among Lepidopterans, Saturniidae, Geometridae and Sphingidae are well represented in the African tropics, with new species regularly described (Brattstroem *et al.*, 2015; Brattstroem *et al.*, 2016; Larsen & Rawlins, 2014; Libert, 2014; Maicher *et al.*, 2016). There, these taxa also include several pest species for plants such as *Dacryodes edulis* (Poligui *et al.*, 2015) and serve as food source for local people (Latham, 2003; Malaise & Lognay, 2003; Michel, 1928; Nsevolo *et al.*, 2016). However, very little is known about them in central Africa (Pierre-Baltus & Pierre, 2000; Pierre, 2001), while biodiversity is increasingly threatened by undergoing significant landscape changes due to road

construction (Blake *et al.*, 2008; Laurance *et al.*, 2006), timber logging (Brugiere, 1998; Laurance *et al.*, 2008; Ndoye & Tieguhong, 2004; Poulsen *et al.*, 2009; Tutin, 2001; Walsh *et al.*, 2004), mining (Edwards *et al.*, 2014; Malhi *et al.*, 2013), industrial plantations (Wich *et al.*, 2014), oil production (Laurance *et al.*, 2008; Malhi *et al.*, 2013; Thibault & Blaney, 2003), and climate change (Malhi *et al.*, 2013; Voje *et al.*, 2009; Willis *et al.*, 2013; Wright, 2010). It is then becoming urgent to find out what Lepidopteran diversity is at stake in the region and how these species are coping with such drastic and rapid changes (Asefi-Najafabady & Saatchi, 2013; Lewis *et al.*, 2013; Mayaux *et al.*, 2013). For this purpose, the mosaic of natural habitats in the Haut-Ogooué province of southeastern Gabon constitutes a unique opportunity to study how Lepidopteran communities might evolve along an ecological gradient with upcoming changes in forest cover in the region (Nana, 1982). The purpose of this study was then to do a preliminary assessment of nocturnal Lepidopteran communities in southeastern Gabon, using both morphological and molecular identifications.

Fig. 1. Sampling locations in southeastern Gabon: Bongoville, Boubou, Souba, and Léconi.

II. METHODS

A. Sampling Sites

The sampling took place between May 22nd and August 3rd, 2014, in the Djouori-Agnili department (Haut-Ogooué province), in south-eastern Gabon (Fig. 1). Four sites (named after the nearest town or village) were selected based on their vegetation types: Bongoville (S1.627°; E13.827°) is located in a mature forest mainly composed of large trees; Boubou (S1.617°; E13.894°) is located at a forest edge and is made up of grasses and large trees; Souba (S1.575°; E14.137°) is located in the savannah and is composed of herbaceous vegetation (medium-sized grasses) and shrubs; and Léconi (S1.525°; E14.290°) is in a forest gallery mainly composed of tall trees and lianas (Fig. 1).

B. Moth Collection

In order to reduce any possible competition with moon light, a total of 24 sampling nights took place during the black moon periods of May 19-30, June 25 to July 6, and July 22 to August 3 of 2014. The protocol used to collect moths was human-operated light-trapping (Chey *et al.*, 1997), which has the advantage over automatic light-trapping of reducing damage to sampled individuals (Axmacher & Fiedler, 2004). At each sampling site, we used an electrical power generator (1500 Watts, KINGmax KM4500DX), a white sheet of 2×2 m, two (2) low-voltage bulbs of 40 watts each (located on either side of the sheet), and a 250-watt mercury vapor lamp (acting as a call lamp) located at a height of about 1.50 m. Collected moths were injected with ammonia or killed in cyanide jars. Finally, moths were stored in glassine envelopes identified with a unique code.

C. Morphological Identification

Back in the laboratory (Biology department, Université des Sciences et Techniques de Masuku, Franceville, Gabon), collected specimens went through two identification phases. The first phase consisted of grouping them into morphospecies sorted within higher taxonomic groups on the basis of the general habitus of the moths. This sorting – usually at family, subfamily or possibly genus level – was verified using images available online from BOLD taxonomy browser (Barcode of Life Datasystems <http://www.boldsystems.org>). A second identification phase was applied to a set of 95 specimens (70 Geometridae, 15 Lymantriinae, and 10 Saturniidae) that were compared to individuals preserved in the reference collections of the Muséum national d'Histoire naturelle (MNHN), Paris (France). These specimens were selected because of the difficulty of discriminating species accurately from a superficial examination of habitus characters only. For the Lymantriinae subfamily, this second identification phase also included genitalia dissection and examination under a binocular lens (LEICA MZ75, KL 1500).

D. Molecular Identification

DNA extraction was carried out on the 95 selected specimens at the MNHN Molecular Systematic Service laboratory following the NucleoSpin96 kit (MACHEREY-NAGEL) protocol. More specifically, a single leg was placed in each well of a 96-well plate (one well left empty as a negative control). Each well received 180 µl of the extraction Buffer T1 and 25 µl of Proteinase K at 0.02 mg/µl. The plate was then incubated at 56 °C overnight. Further DNA extraction steps were completed automatically using an Eppendorf epMotion 5075.

Then, a fragment of 658 base pairs of the 5' end region of the mitochondrial cytochrome oxidase I (COI) gene was

amplified by Polymerase Chain Reaction (PCR). The amplification mix was prepared in a 5 ml Eppendorf tube containing 1650 µl of distilled water, 200 µl of Buffer 2 (10 mM), 80 µl of dNTP (6.6 mM), and 32 µl of each primer (LEP-F1, 5'-ATTCAACCAATCATAAAGATAT-3'; LEP-R1, 5'-TAAACTTCTGGATGTCCAAAAA-3') (Hebert *et al.*, 2004). After homogenization, 1.5 µl of DNA from each sample was added to each of the 95 wells containing 20 µl of the amplification mix. PCR took place in a thermocycler (Bio-RAD, C9000-Touch, CFX96 Real TimeSystem) with the following cycles: (1) hot start for 1 min at 94 °C; (2) denaturation for 40 seconds at 94 °C; (3) hybridization for 40 seconds at 45 °C; (4) expansion for 1 minute at 72 °C; (5) 5 cycles of steps 2 to 4; (6) denaturation for 40 seconds at 94 °C; (7) hybridization for 40 seconds at 51 °C; (8) extension for 1 minute at 72 °C; (9) 35 cycles of steps 7 to 8; (10) final extension for 10 minutes at 72 °C; and indefinite storage time at 12 °C before storage of PCR products at -9 °C in a freezer (SJ-14T-G2S). Visualization of PCR products was carried out on a 2% agarose gel. Migration was performed at 100 V for 15 min. The result of DNA migration was photographed with a digital camera (SAMSUNG, ES90) in order to visualize the quality and the size of the DNA fragments.

After sequencing of the PCR products by EUROFINs (<https://www.eurofins.fr/>), we used CodonCode (CodonCode Corp., Centerville, USA) for trimming primers, contig assembly and sequence editing. The sequences were then aligned using BioEdit version V7.2.5 and uploaded, along with all specimen data and images into the project "Lepidoptera of the Plateau Bateke region, Gabon" (LPBTK) on BOLD. Analyses of genetic distances and their visualization using Neighbor-Joining (NJ) were carried out using the analytical tools implemented in BOLD (default settings, using the K2P (Kimura 2-parameter) model of evolution (Kimura, 1980).

III. RESULTS

A. Morphological Identification

A total of 2418 specimens of nocturnal Lepidoptera were collected in the four different sites and during the three sampling sessions (Table I). The highest number of nocturnal Lepidoptera was collected in the first session of May (n = 1040), while the lowest number was collected in the third session of July-August (n = 430).

The site with the highest number of collected nocturnal Lepidoptera was Bongoville (n = 643), while the site with the lowest number was Souba (n = 603). This resulted in a significant relationship between the number of collected nocturnal Lepidoptera specimens per sampling session and locations ($X^2 = 66.261$; $P = 2.383e-12$).

Of the 14 identified families, the Geometridae were the most abundant (n = 938) (Table I). There was a significant relationship between the number of collected nocturnal Lepidoptera specimens per family and sampling locations ($P < 1e-7$).

TABLE I: NUMBER OF SPECIMENS PER FAMILY COLLECTED IN EACH SAMPLING SITES AND THEIR TOTALS

Families	Bongoville	Boubou	Souba	Léconi	Total
Aganainae	8	3	0	0	11
Arctiinae	1	3	2	8	14
Chrysopolominae	16	0	0	9	25
Crambidae	22	0	20	36	78
Eupterotidae	5	0	0	0	5
Geometridae	267	254	134	283	938
Cossidae	0	0	0	3	3
Lasiocampidae	26	62	13	30	131
Limacodidae	0	9	0	1	10
Lymantriinae	28	24	0	57	109
Noctuidae	6	3	2	4	15
Nymphalidae	0	0	1	0	1
Saturniidae	45	36	0	25	106
Sphingidae	184	152	372	102	810
Unidentified	35	57	29	41	162
Total	643	603	573	599	2418

Fig. 2. Two examples of genitalia observed in selected Lymantriinae specimens.

Of the 95 specimens (70 Geometridae, 15 Lymantriidae, and 10 Saturniidae) further morphologically analyzed at the MNHN, these were grouped in 10 Geometridae, three (3) Saturniidae, and one (1) Lymantriinae morphospecies (Supplementary Table I). After careful observation, selected Saturniidae and Lymantriinae specimens were identified as belonging to genera *Imbrasia* and *Leucoma* or *Euproctis*, respectively.

While a superficial comparison of the moth habitus had led to the recognition of a single morphospecies among the 11 selected specimens of Lymantriinae, our comparative analysis of male genitalia morphology allowed for the discrimination of nine (9) morphospecies. This was accomplished by considering morphological variation of the general shape and size of the valves and of the uncus (Fig. 2), as well as that of the aedeagus.

B. Molecular Identification

We obtained DNA barcode sequences for 91 of the 95 specimens sampled for DNA barcoding. All are publicly available in project LEPBTK on BOLD, as well as in GenBank (see BOLD project for GenBank accession numbers). Using Barcode Index Numbers (BINs; (Ratnasingham & Hebert, 2013) as a molecular proxy for species (as in (Hausmann *et al.*, 2013; Ortiz *et al.*, 2017)), our results suggest that these 91 samples (see Suppl. Fig. 1 for a visualization of these results as a NJ tree) may represent a total of 52 distinct species. In Lymantriinae, all nine species discriminated by their male genitalia (see above) formed unique and distinct genetic clusters (BINs). In Geometridae, the 66 DNA barcoded records comprised representatives of the 10 initially recognized morphospecies; they formed 41 distinct BINs.

IV. DISCUSSION AND CONCLUSION

This assessment of nocturnal Lepidopteran communities in southeastern Gabon provided a preliminary glimpse into the diversity of these moths in the Plateaux Batéké region. Estimates of species diversity carried out in the field, using a morphospecies approach, were supplemented, for a subset of the moths sampled, by a more thorough integrative approach combining morphology and DNA barcoding. This clearly revealed that preliminary sorting of specimens by morphospecies resulted in a strong underestimation of the actual species diversity, as evidenced by our subset of 14 morphospecies that turned out to represent 52 distinct BINs; a molecular proxy to species diversity. The diversity at family or subfamily level was higher in the site located within the continuous forest (namely, the Bongoville site, see Table I), probably because of a plant diversity higher than in the other sampled habitat types (Hawes *et al.*, 2009).

Nevertheless, the four sampling locations were rather similar in terms of the total number of collected specimens and families or subfamilies. Further studies are needed however to better document similarities and differences in diversity, composition, and structure of moth communities in the region. As demonstrated here, these studies should ideally include a thorough morphological analysis or the use of DNA barcodes to assess species diversity, or a combination of both methods.

APPENDIX

TABLE I: NUMBER OF SPECIMENS PER MORPHOSPECIES, SAMPLED SITES AND THEIR TOTALS OUT OF THE 95 SPECIMENS ANALYZED BY DNA BARCODING AT THE MUSÉUM NATIONAL D'HISTOIRE NATURELLE (MNHN)

Families	Morpho-species	Bongo-ville	Boubou	Souba	Léconi	Total
Geometridae	Geo1	6	0	2	0	8
	Geo2	1	3	1	0	5
	Geo3	4	2	0	0	6
	Geo4	3	3	1	1	8
	Geo5	3	4	0	0	7
	Geo6	2	0	2	0	4
	Geo7	1	2	0	0	3
	Geo8	4	6	4	0	14
	Geo9	1	1	2	0	4
	Geo10	4	7	0	0	11
Lymantriidae	Lym1	5	3	7	0	15
Saturniidae	Sat1	1	0	3	0	4
	Sat2	2	1	0	0	3
	Sat3	0	1	2	0	3
Total		37	33	24	1	95

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

Supplementary Fig. 1. Neighbour Joining tree of 91 DNA barcode sequences generated for Lepidoptera (Geometridae, Lymantriidae and Saturniidae) of the Batéké plateau (southeastern Gabon).

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