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Short Communication

Morpho-Geometric Analysis of Eight Grass Mouse Species of the Genus *Lemniscomys* (Rodentia: Muridae)

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Authors' Contributions
IBS and ABI conceived and designed the study. They also acquired, analysed and interpreted the data. MC and SN participated in drafting and revising the article.

Key words
Lemniscomys, Morphometric geometrics, Africa, Rodents, Skull

ABSTRACT

Lemniscomys are exclusively African rodents. This study deals with morphs-genetic analysis of eight species *viz. Lemniscomys barbarus*, *L. bellieri*, *L. griselda*, *L. limulus*, *L. macculus*, *L. rosalia*, *L. striatus* and *L. zebra*; which have practically similar external morphologies although they spread in completely different geographical areas. The approach adopted was to identify landmarks on the skulls of all specimens using the tpsDig software, and then analyzing them through the program MorphoJ. Our results show that *L. griselda* has the largest skull, whereas *L. zebra* has the smallest. *L. griselda* and *L. rosalia* have greater breadth of the braincase, length of the nasals and length of the tympanic bulla, than the other species. This is probably related to a phenotypic evolution due to selective pressure, as is also the case in the genus *Gerbillus*.

Research in systematics has benefited from several new techniques developed in recent years, including geometric morphometric analysis. It was developed to address the problems of systematic relationships and evolution, and made significant contributions to studies related to rodents which constitute the majority of the mammalian biodiversity in Africa (Denys *et al*., 2003). However, recent studies on genus *Lemniscomys* (Trouessart, 1881) have exclusively focused on the biogeographical, phylogenetic, chromosomal and molecular analysis (Castaglia *et al*., 2002; Nicolas *et al*., 2008; Mboumba *et al*., 2012) at the expense of morphometric analysis. This genus is one of the more diverse groups of rodents; its distribution is exclusively African and covers much of the continent with the exception of the Sahara which is a major ecological barrier. The genus *Lemniscomys* includes eleven species (Kingdon *et al*., 2013), which are characterized by their external appearance, in fact the color of their fur consists of one or more longitudinal stripes hence the term striped mouse. In the present study, we will focus on geometric morpho-metric analysis of eight species, which have close external morphologies despite occurring in different geographic areas (Carlton and Van der Straten, 1997) and possess dissimilar chromosome sets (Castaglia and Oguge, 2008).

Material and methods

A total of 200 adult specimens were analyzed, representing eight species *L. barbarus* (Linnaeus, 1766), *L. bellieri* (Van der Straeten, 1975), *L. griselda* (Thomas, 1904), *L. limulus* (Thomas, 1910), *L. macculus* (Thomas and Wroughton, 1910), *L. rosalia* (Thomas, 1904), *L. striatus* (Linnaeus, 1758) and *L. zebra* (Heuglin, 1864). Each species is represented by 25 specimens, they are deposited in Institut Royale des Sciences Naturelles de Belgique (IRSNB), Musé Royal de l'Afrique Centrale (MRAC) and Faculté des Sciences de Tunis (FST) in the Research Unit "Biodiversité et Biologie des Populations" (Supplementary Table I). The deposited specimens in the two museums mentioned above were identified according to Van der Straeten in his previous works (Carlton and Van der Straeten, 1997; Van der Straeten and Verheyen, 1980).

The skulls of all specimens were photographed on the ventral side using a Canon PowerShot A2200 HD camera (14.1 MP resolution). Using the software tpsDig, version 1.40 (Rohlf, 2009) 20 landmarks are identified on the skull images (Fig. 1).

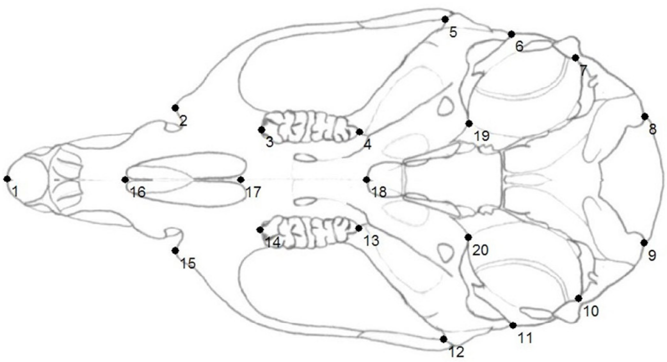

Fig. 1. Placement of the collected landmarks
1, tip of the nasal; 2, 15, inferior margin of infraorbital foramen; 3, 14, anterior extremity of molar row; 4, 13, posterior extremity of molar row; 5, 12, back of zygomatic notch; 6, 11, tympanic bulla at the posterior border of the external auditory meatus; 7, 10, posterior extremity of the tympanic bulla; 8, 9, posterior intersection between foramen magnum and occipital condyle; 16, anterior extremity of foramen; 17, posterior extremity of foramen; 18, aAnterior limit of mesopterygoid fossa; 19, 20, junction between tympanic bulla and pterygoid process.

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The configurations of landmarks were analyzed by the MorphoJ program version 1.05f (Klingenberg, 2013). The average size of a skull was obtained from the square root of the sum of squared distances between landmarks and the center of gravity (or centroid) of the skull (Bookstein, 1991). The difference in average size of skulls between species is visualized by box plots. Differences in the shape of skulls between species are visualized through the Canonical Variate Analysis (CVA). Finally, Unweighted Pair Group Method with Average (UPGMA) was computed from the Procrustes distances obtained through ventral side configurations. All statistical analyses were performed using the PAST software, version 2.17 (Rohlf, 2009).

Results

We noticed a significant variation in the size of skulls (Fig. 2), Turkey HSD test suggests that *L. griselda* have the largest skulls (P <0.001) whereas *L. zebra* presents the smallest ones (P <0.001). The other species *L. barbarus*, *L. bellieri*, *L. limulus*, *L. macculus*, *L. rosalia* and *L. striatus* show intermediate sizes.

The variation in the shape of skulls is not related to the size of the skulls. The Canonical Variate Analysis (CVA; Fig. 3) corresponding to the ventral side configuration shows us a significant difference in the shape of skulls between species (Manova: Wilks' λ = 0.0063; F = 14.29; P <0.001), the first 2 axes of the CVA absorbing 73.024% of variances, axis 1 (55.456%) allows discrimination of *L. griselda*, *L. linulus* and *L. rosalia* which are located in the positive part of the first axis, *L. barbarus*, *L. macculus*, and *L. zebra* are located in the negative part, whereas *L. bellieri* and *L. striatus* are located in the intermediate part with a substantial overlap. Axis 2 (17.568%) of the CVA shows significant overlapping between species, however it allows the discrimination between *L. barbarus* and *L. zebra*.

The variation on the axis 1 of the CVA focuses on landmarks n° 1, 16, 6, 11, 19 and 20 (Fig. 4), which menas that the most discriminating factors are the breadth of the braincase (landmarks n° 6 and 11), length of nasals (landmarks n° 1 and 16) and length of tympanic bulla (landmarks n° 19 and 20). The latter is more developed among *L. rosalia* and *L. griselda* than among the other species.

The UPGMA tree (Fig. 5) computed from the obtained Procrustes distances reveals the existence of two groups: the first consists of *L. barbarus*; *L. bellieri*; *L. macculus* and *L. zebra*, the second is formed by *L. linulus* and *L. rosalia*; *L. striatus* has a central position, whereas *L. griselda* has a basal position in the UPGMA tree.

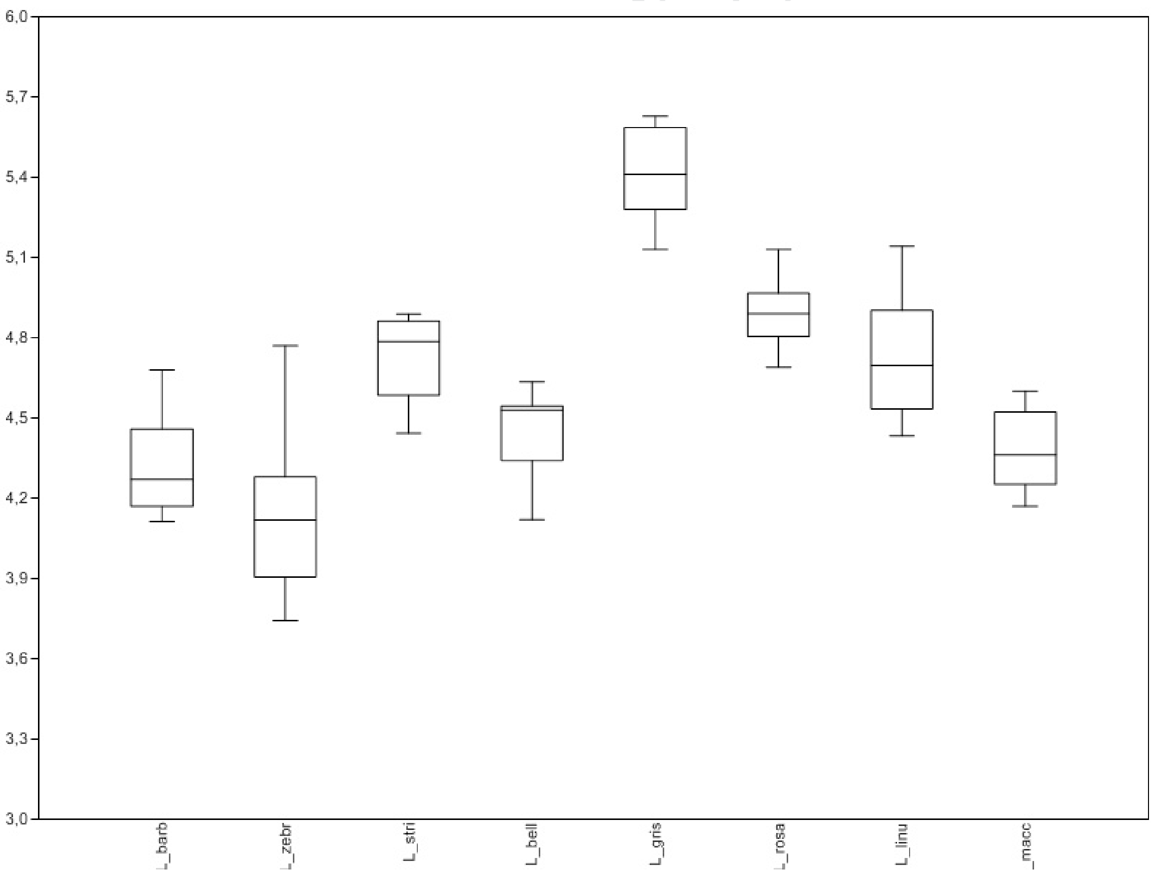

Fig. 2. Box plot showing the average of centroid size based on ventral configurations of each species. Box margin are the 25th and 75th percentiles, bars extend to 5th and 95th percentiles, the inner line represents the median.

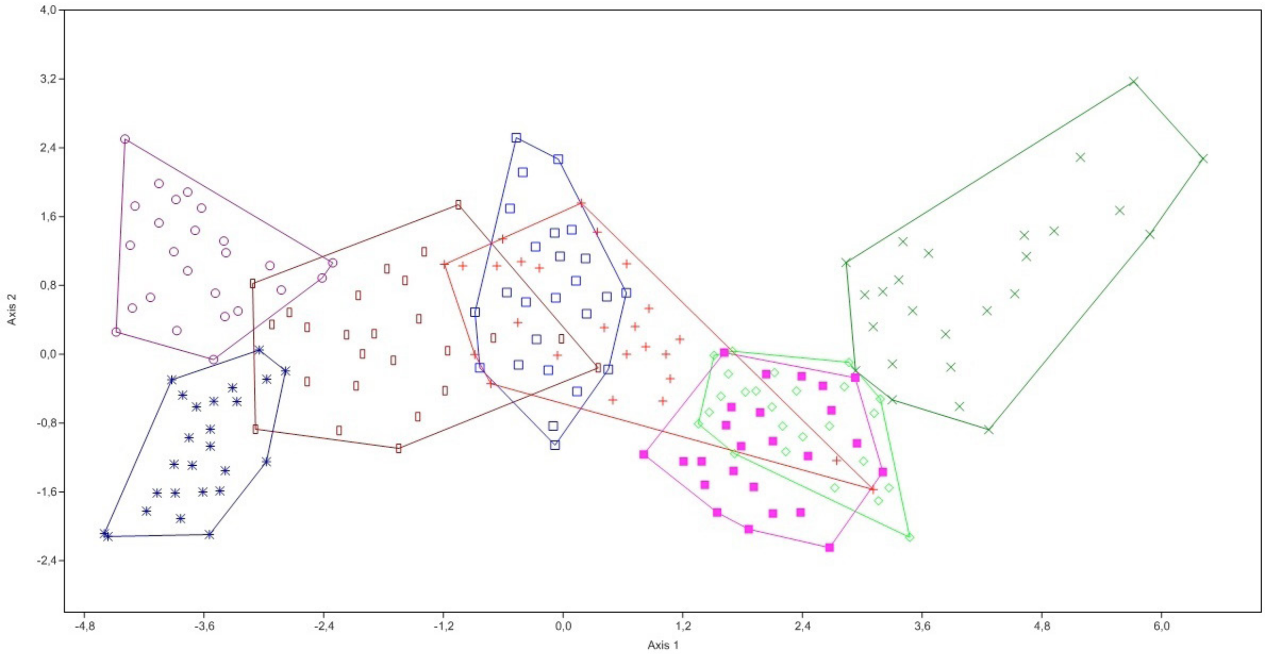

Fig. 3. Canonical Variate Analysis (CVA) of ventral configurations; ○ *L. barbarus*; □ *L. bellieri*; × *L. griselda*; ■ *L. linulus*; ▯ *L. macculus*; ◇ *L. rosalia*; + *L. striatus*; ✳ *L. zebra*.

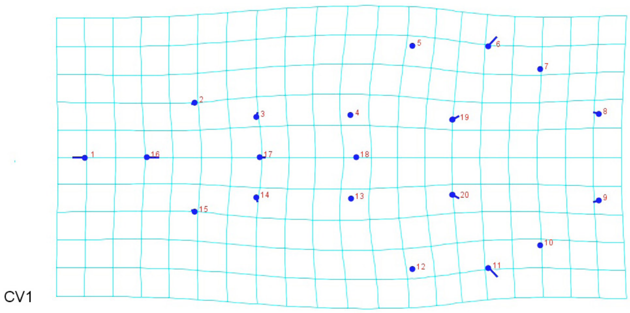

Fig. 4. Variation of landmarks on axis 1 of the CVA.

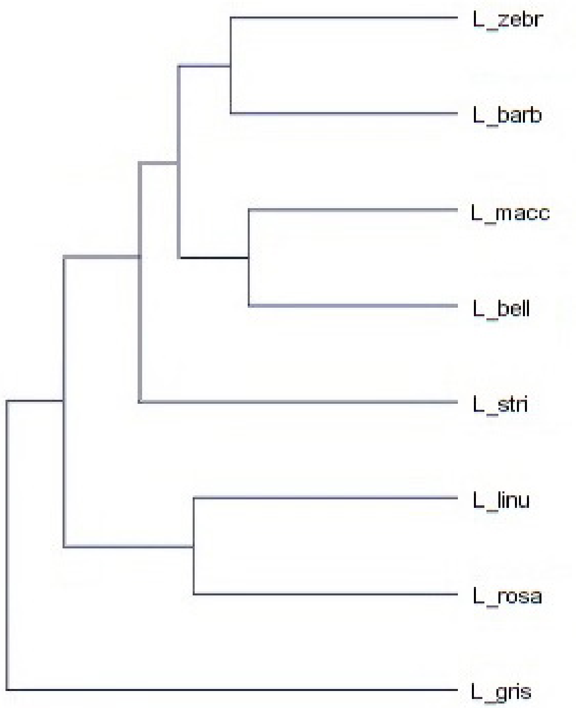

Fig. 5. UPGMA based on Procrustes distances for ventral configurations of skulls.

Discussion

Geometric morphometric analysis is used for the first time on the genus *Lemniscomys*. Our results are close to those found by Van der Straeten and Verheyen (1980), despite having used multivariate analysis methods based on cranial measurements. However, recent molecular studies of the genus *Lemniscomys* show that *L. rosalia*, *L. striatus* and *L. zebra* have the same ancestry (Castaglia et al., 2002), which differs from the results found by morphometric analysis mentioned above and the present analysis. The difference, in this case, between molecular and morphometric analysis can be explained by a high rate of phenotypic evolution, this suggests the action of different selective pressures or functional constraints in the morphological evolution of the genus *Lemniscomys*, as in the case of the genus *Gerbillus* (Abiath, 2002; Abiath et al., 2010). Our results show that close species morphologically on the cranial level are more or less sympatric species such as *L. macculus* and *L. zebra* (Eastern Africa) or *L. griselda*and *L. rosalia* (Zambia) covering the same geographical areas (Kingdon *et al*., 2013).

Conclusion

The morpho-geometric approach applied on eight species of the genus *Lemniscomys* shows that the closest species on a cranial level can have dissimilar cytogenetic sets. It is due to a high rate of phenotypic evolution that can surpass the molecular counterpart. It would be interesting to explore this difference between morpho-geometric and cytogenetic results with further molecular studies of this genus.

Acknowledgements

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Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of the manuscript.

Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2017.x.x.xxx.xxxxxxxxx

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Supplementary Material

Short Communication

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0030-9923/2016/0004-1161 $ 8.00/0

References of the biological material examined

L. barbarus: IRSN-40167; IRSN-40168; IRSN-40169; IRSN-40170; IRSN-40171;
IRSN-40172; IRSN-40173; IRSN-40174; IRSN-40175; MRAC-b4-007-M0001;
MRAC-b4-007-M0002; MRAC-b4-007-M0003; MRAC-b4-007-M0004; MRAC-b4-007-M0005;
MRAC-b4-007-M0006; MRAC-b4-007-M0007; FST-01; FST-02; FST-03; FST-04; FST-05; FST-06; FST-07; FST-08; FST-09.

L. bellieri: MRAC-077-054-M0125; MRAC-077-054-M0124; MRAC-077-054-M0123;
MRAC-077-054-M0111; MRAC-077-054-M0110; MRAC-077-054-M0109;
MRAC-077-054-M0108; MRAC-077-054-M0107; MRAC-077-054-M0106;
MRAC-077-054-M0105; MRAC-077-054-M0104; MRAC-077-054-M0103;
MRAC-077-054-M0102; MRAC-077-054-M0101; MRAC-077-054-M0100;
MRAC-077-054-M0099; MRAC-077-054-M0098; MRAC-077-054-M0097;
MRAC-077-054-M0096; MRAC-077-054-M0095; MRAC-077-054-M0094;
MRAC-077-054-M0093; MRAC-077-054-M0092; MRAC-077-054-M0091;
MRAC-077-054-M0090.

L. griselda: IRSN-11115; IRSN-11116; IRSN-11117; IRSN-11118; IRSN-11119;
IRSN-11120; IRSN-39512; IRSN-39513; IRSN-03119; IRSN-16221; IRSN-16222;
IRSN-16223; IRSN-16224; IRSN-16225; IRSN-16226; IRSN-16227; IRSN-16228;
IRSN-16229; IRSN-16230; IRSN-16231; MRAC-078-034-M0050; MRAC-078-034-M0051;
MRAC-078-034-M0052; MRAC-078-034-M0058; MRAC-078-034-M0063.

L. linulus: MRAC-093-012-M0403; MRAC-093-012-M0423; MRAC-080-037-M0078;
MRAC-080-037-M0081; MRAC-080-037-M0080; MRAC-080-037-M0076;
MRAC-080-037-M0075; MRAC-080-037-M0073; MRAC-080-037-M0079;
MRAC-080-037-M0077; MRAC-080-037-M0074; MRAC-080-037-M0066;
MRAC-080-037-M0067; MRAC-080-037-M0068; MRAC-080-037-M0065;
MRAC-080-037-M0034; MRAC-080-037-M0035; MRAC-080-037-M0036;
MRAC-080-037-M0031; MRAC-080-037-M0030; MRAC-080-037-M0028;
MRAC-080-037-M0029; MRAC-080-037-M0032; MRAC-080-037-M0033;
MRAC-080-037-M0024.

L. macculus: MRAC-082-011-M0628; MRAC-082-011-M0624; MRAC-082-011-M0623;
MRAC-082-011-M0626; MRAC-082-011-M0625; MRAC-082-011-M0627;
MRAC-078-039-M0372; MRAC-078-039-M0371; MRAC-078-039-M0363;
MRAC-078-039-M0353; MRAC-078-039-M0362; MRAC-078-039-M0355;
MRAC-078-039-M0364; MRAC-078-039-M0354; MRAC-078-039-M0357;
MRAC-078-039-M0356; MRAC-078-039-M0366; MRAC-078-039-M0365;

MRAC-078-039-M0360; MRAC-078-039-M0359; MRAC-078-039-M0368;
MRAC-078-039-M0369; MRAC-078-039-M0370; MRAC-078-039-M0361;
MRAC-078-039-M0358

L. rosalia: MRAC-096-036-M4192; MRAC-096-036-M4193; MRAC-096-036-M4194;
MRAC-096-036-M4195; MRAC-096-036-M4196; MRAC-096-036-M4214;
MRAC-096-036-M4215; MRAC-096-036-M4197; MRAC-096-036-M4198;
MRAC-096-036-M4199; MRAC-096-036-M4200; MRAC-096-036-M4201;
MRAC-096-036-M4202; MRAC-096-036-M4203; MRAC-096-036-M4204;
MRAC-096-036-M4205; MRAC-096-036-M4206; MRAC-096-036-M4207;
MRAC-096-036-M4208; MRAC-096-036-M4209; MRAC-096-036-M4210;
MRAC-096-036-M4211; MRAC-096-036-M4212; MRAC-096-036-M4213;
MRAC-096-036-M4216.

L. striatus: IRSN-17054; IRSN-17055; IRSN-17056; IRSN-17052; IRSN-17053;
IRSN-16740; IRSN-16739; IRSN-16738; IRSN-16742; IRSN-16741; IRSN-12883;
IRSN-12884; IRSN-20888; IRSN-13432; IRSN-13431; IRSN-13438; IRSN-13437;
IRSN-13435; IRSN-13433; IRSN-13436; IRSN-13434; IRSN-04686; IRSN-04684;
IRSN-04685; IRSN-04683.

L. zebra: MRAC-096-037-M2731; MRAC-096-037-M2732; MRAC-096-037-M2734;
MRAC-096-037-M2735; MRAC-096-037-M2736; MRAC-096-037-M2737;
MRAC-096-037-M2738; MRAC-073-015-M0920; MRAC-073-015-M0919;
MRAC-073-015-M0918; MRAC-073-015-M0917; MRAC-073-015-M0916;
MRAC-097-078-M0508; MRAC-097-078-M0509; MRAC-097-078-M0510;
MRAC-097-078-M0511; MRAC-097-078-M0512; MRAC-097-078-M0513;
MRAC-097-078-M0514; MRAC-097-078-M0515; MRAC-097-078-M0516;
MRAC-097-078-M0517; MRAC-097-078-M0518; MRAC-097-078-M0519;
MRAC-097-078-M0520.

Supplementary Table I.- List of the specimens included in the analysis

Species	Country	Locality	No of specimens	Institute of deposit
L. barbarus	Tunisia	Aïn Ghazel,	3	MRAC
		Borj El Amri	3	MRAC
		Lansarine	9	FST
			9	IRSNB
			1	MRAC
L. zebra	Tanzania	Tagamenda	2	MRAC
		Gerodom	4	MRAC
		Katesh	1	MRAC
	Cameroun	Sir	5	MRAC
	Togo	Naso	13	MRAC
L. striatus	Ivory Coast	Dabou	3	IRSNB
		Toumodi	2	IRSNB
	Liberia	Nimba	3	IRSNB
		Yekepa	2	IRSNB
	D. R. Congo	Gangala-na-Bodio	2	IRSNB
		Kisangani	1	IRSNB
		Nord-Kivu	12	IRSNB
L. bellieri	Ivory Coast	Lomto	14	MRAC
		Telekio	6	MRAC
		Kafiné	5	MRAC
L. griselda	D. R. Congo	National Parc of Upenba	6	IRSNB
		Nord-Kivu	1	IRSNB
	Rwanda	National Parc of Akagera	12	IRSNB
	Tanzania	National Parc of Serengeti	1	IRSNB
	Angola	Guimbango	3	MRAC
		Chilambo	1	MRAC
		Guiteve	1	MRAC
L. rosalia	Tanzania	Morogoro	7	MRAC
		Dakawa	18	MRAC
L. linulus	Ivory Coast	Bouna	13	MRAC
		Ouango Fitini	12	MRAC
L. macculus	D. R. Congo	Sinda	6	MRAC
		National Parc of Garamba	19	MRAC

MRAC: Musé Royal d'Afrique Centrale.
IRSNB: Institue Royale des Sciences Naturelles de Belgique.
FST: Faculté des Sciences de Tunis.