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Disputed taxonomy classification of sportive lemurs (*Lepilemur*) in NW Madagascar

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The years 2006 and 2007 have seen a massive rise in diversity on the species level of sportive lemurs (*Lepilemur*). The number of species has increased from eight to more than 20 species (Andriaholinirina *et al.*, 2006; Louis *et al.*, 2006; Rabarivola *et al.*, 2006; Craul *et al.*, 2007). Since many of the new forms have been described solely on mtDNA sequence variation, the validity of the

proposed new species has been questioned by Tattersall (2007). Here we will not discuss the usefulness and pitfalls of species delimitation based solely on mtDNA markers. In this paper we will comment on what appears to be an artificially inflated number of species in the former range of *Lepilemur dorsalis* due to the lack of knowledge about the exact type locality of *L. dorsalis* and to the incomparability of DNA markers used by different authors.

The holotype of *Lepilemur dorsalis* Gray, 1871 is in the British Museum of Natural History and its type locality is specified as "NW Madagascar?" (Jenkins, 1987). Where exactly it comes from is apparently not known. The same is the case for the name *Lepilemur grandidieri* Forsyth Major, 1894, a name which is traditionally regarded as a synonym of *Lepilemur dorsalis* but which must also be taken into account when describing putative new species.

In what follows, when we refer to "the former range of *L. dorsalis*", we mean the area mapped for the taxon recognised under that name by Petter *et al.* (1977) and Tattersall (1982).

Recent molecular genetic and cytogenetic studies depict the situation of *Lepilemur* in the Sambirano region of NW Madagascar as follows (Fig. 1; Tab. 1).

1. Andriaholinirina *et al.* (2006) identified two *Lepilemur* taxa within the former range of *L. dorsalis* and one, actually, from just beyond it. *L. sahamalazensis* was a new species from Sahamalaza (just beyond the southern border of the former range of the *L. dorsalis*). Samples from the island of Nosy Be and the opposite region of Manehoka formed a separate, distinctive clade which they considered to be the true *L. dorsalis*. *L. dorsalis* is found only in Inter River System IV (IRS). We refer to this concept as *L. dorsalis* (1).
2. Louis *et al.* (2006) analyzed samples also from Nosy Be, but not from Manehoka. Their Nosy Be specimens were described as a new species, *L. tymerlachsoni*, because they considered specimens from south of the Sambirano (Antafondro and Manongarivo) as *L. dorsalis*. *L. dorsalis* is found only in IRS V. We refer to this concept as *L. dorsalis* (2).
3. Rabarivola *et al.* (2006) described a new species from the Ampasindava peninsula, *L. mittermeieri*. They adopt the concept of Andriaholinirina *et al.* (2006), i.e. *L. dorsalis* (1).
4. Craul *et al.* (2007) considered specimens within the former range of *L. dorsalis* as *L. dorsalis* but suggest that two subspecies exist north and south of the Sambirano river (IRS V and VI). We refer to this concept as *L. dorsalis* (3).

Additionally, Louis *et al.* (2006) described a new species from Anjiamangirana, in the former range of *L. edwardsi* as *L. grewcocki*, whereas Craul *et al.* (2007) described specimens from nearly the same locality as *L. manasamody*. Craul *et al.* (2007) added a second new species to the list from Ambodimahabibo, *L. otto*.

The ranges of the various taxa are partly congruent with the centres of endemism of the Watershed model (Wilmé *et al.*, 2006) and the interfluvial regions of the Inter River System model (Olivieri *et al.*, 2007). However, two taxa have been described in each of three interfluvial regions; *L. tymerlachsoni* and *L. dorsalis* (1) for Nosy Be and Manehoka (IRS VI), *L. mittermeieri* and *L. dorsalis* (2) from Ampasindava and Manongarivo

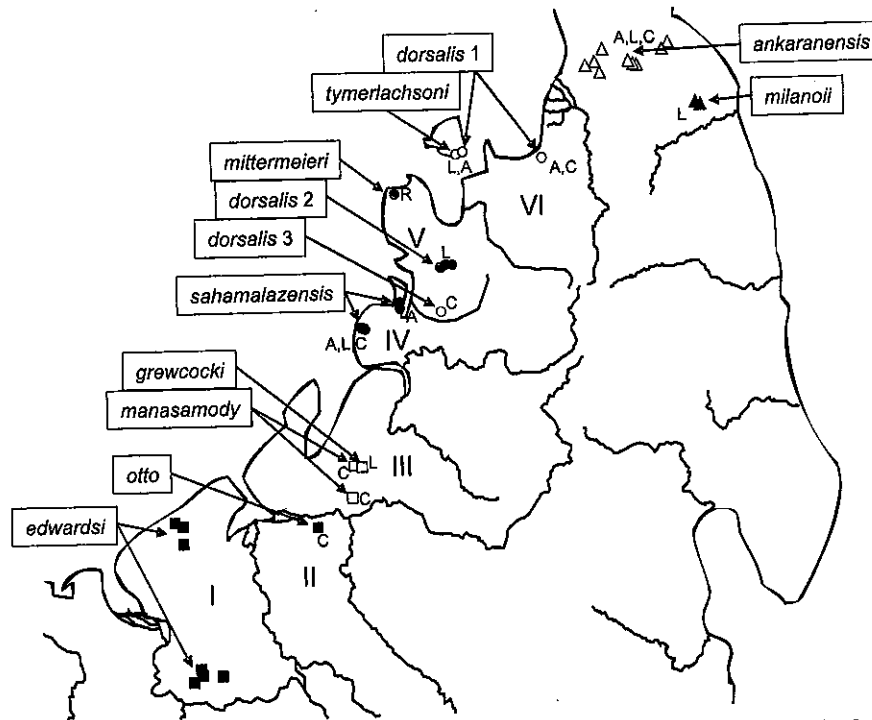


Fig. 1: *Lepilemur* sampling sites in NW Madagascar. Sampling sites of: A = Andriaholinirina *et al.*, 2006; R = Rabarivola *et al.*, 2006; L = Louis *et al.*, 2006; C = Craul *et al.*, 2007; I – VI Inter River Systems.

Table 1: Origins of *Lepilemur* specimens.

Site; IRS	Coordinates in decimal degrees	Andriaholinirina <i>et al.</i> , 2006	Rabarivola <i>et al.</i> , 2006	Louis <i>et al.</i> , 2006	Craul <i>et al.</i> , 2007
Nosy Be; VI	48.33° E, 13.39° S	<i>dorsalis</i>		<i>tymerlachsoni</i>	<i>dorsalis</i>
Manehoka; IV	48.80° E, 13.43° S	<i>dorsalis</i>			<i>dorsalis</i>
Ampasindava; V	47.90° E, 13.60° S		<i>mittermeieri</i>		
Antafondro; V	48.20° E, 14.04° S			<i>dorsalis</i>	
Manongarivo; V	48.27° E, 14.02° S			<i>dorsalis</i>	
Mahilaka; V	48.20° E, 14.29° S				<i>dorsalis</i>
Sahamalaza; IV	47.97° E, 14.23° S	<i>sahamalazensis</i>			
Ankarafa; IV	47.75° E, 14.37° S			<i>sahamalazensis</i>	<i>sahamalazensis</i>
Anjiamangirana Class. Forest; III	47.72° E, 15.15° S			<i>grewcocki</i>	<i>manasamody</i>

(IRS V), and *L. grewcocki* and *L. manasamody* from Anjiamangirana and Ambongabe (IRS III). Two questions arise: (1) are the taxa from the same biogeographical region really different taxa or do they constitute synonyms, and (2), of more relevance to the present paper, which of the two *dorsalis* taxa are correctly named, and which might correspond to *L. grandidieri* Forsyth Major, 1894?

Methods

Since sequence data from *L. manasamody* and *L. otto*, and from the specimens from Mahilaka (possibly also *L. dorsalis* (2)), are not available at the moment, these taxa are not included in the analysis but have been included provisionally (graphically) in a phylogenetic tree reconstruction. Data of the Louis *et al.* 2006 specimens are derived from the NCBI GenBank.

We sequenced specimens from Ankarana, Nosy Be, Manehoka, Ampasindava, Sahamalaza and Ampijoroa. Animals were captured using blowpipe projection. Skin

samples were cut off under general anaesthesia with a 2 mg/kg injection of ketamine solution (Ketalar® Parke-Davis) and preserved in liquid nitrogen.

The extraction of DNA was performed with QIAamp DNA Minikit according to the manufacturer procedures. Amplification of a fragment about 550 bp long of the mitochondrial D-loop region was generated via PCR with primers as described (Wyner *et al.*, 2000; Baker *et al.*, 1993). Reactions were performed in a Perkin Elmer Cetus DNA thermocycler 480 as follows: predenaturation (10 min at 94° C) and 35 cycles each consisting of denaturation (1 min at 94° C), annealing (1 min at 54° C) and extension (1 min 30 s at 72° C), followed by a final extension step (10 min at 72° C). The results of the amplifications were checked on 1 % agarose gels and the PCR products cleaned with the Qiagen PCR Purification kit. PCR products were sequenced from both directions on an automated ABI PRISM sequencer with the BigDye Terminator Cycle Sequencing kit. Due to the low number of indels, sequences were easily aligned by eye. The final dataset included sequences from 22 sportive lemurs from NW Madagascar as well as from one specimen of *L. leucopus*, which was used for outgroup purposes. Pairwise genetic distances (d) were calculated with Mega 3.1 (Kumar *et al.*, 2004). Before

reconstructing phylogenetic relationships, poorly aligned positions and gaps were removed with the Gblocks software (Castresana, 2000), which reduced the final dataset to 505 bp. Phylogenies were generated with the maximum-likelihood (ML), neighbor-joining (NJ) and maximum-parsimony (MP) algorithms as implemented in PAUP 4.0b10 (Swofford, 2002) and TREEPUZZLE 5.0 (Strimmer and von Haeseler, 1996). For MP analyses, all characters were treated as unordered and equally weighted throughout. A heuristic search was performed with the maximum number of trees set to 100. NJ and ML trees were constructed with the HKY + I (= 0.6202) + Γ (= 0.5774) model of sequence evolution as it was selected as best-fitting model with MODELTEST 3.06 (Posada and Crandall, 1998) as well as with standard models. Relative support of internal nodes was performed by bootstrap analyses with 1,000 replications (MP, NJ), or by the quartet puzzling support values on the basis of 1,000 puzzling steps (ML).

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