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New Evidence of the Semi-Aquatic Nimba Otter Shrew (*Micropotamogale lamottei*) at Mount Nimba and in the Putu Range of Liberia – Uncertain Future for an Evolutionary Distinct and Globally Endangered (EDGE) Species in the Face of Recent Industrial Developments

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Abstract: New records of the endemic semi-aquatic Nimba Otter Shrew *Micropotamogale lamottei* (Tenrecidae, Potamogalinae) were obtained during recent Environmental Impact Assessments (EIAs) in Liberia. We discuss the future of this Evolutionary Distinct and Globally Endangered (EDGE) species in the face of large-scale landscape transformations. In an attempt to assess genetic diversity and genetic differentiation of *M. lamottei* throughout its distributional range, we examined two mitochondrial loci (cytochrome *b* and 12S rRNA) from five samples of Nimba Otter Shrews at three localities in Liberia and compared the 12S sequences with a GenBank sequence from Côte d'Ivoire. While all six individuals examined had unique mitochondrial haplotypes, the sequence divergence among these haplotypes was less than 1 %. However, the F_{ST} values indicated substantial differences between East Nimba and the other two populations. Recognizing that the genetic results are based on very small samples, the data suggest that the three populations experienced sufficient gene flow in the past, preventing sequence divergence, but currently there is substantially reduced gene flow with the East Nimba population. More targeted surveys in the Upper Guinea Region are recommended for this elusive species using aquatic traps in combination with an environmental DNA (eDNA) approach.

Keywords: *mammals, Afrotheria, Tenrecidae, Upper Guinea, West Africa, conservation*

Otter shrews (subfamily Potamogalinae) are unrelated to true shrews (family Soricidae, order Soricomopha), but belong to the African family Tenrecidae. They are part of a superordinal clade of anatomically and biologically quite dissimilar mammals of African origin, which, based on strong molecular evidence, are now united in the Supercohort Afrotheria (Stanhope et al. 1998b; Madsen et al. 2001; Kingdon et al. 2013). Within the Afrotheria, the Tenrecidae are combined with the golden moles (Chrysochloridae) in the African, or Gondwanan order called Afrosoricida (Stanhope et al. 1998a) or Tenrecoidea (Malia et al. 2002; Poux et al. 2008; Asher and Helgen 2010). Because of this independent African origin, golden moles present a convergent adaptive radiation to true moles (Talpidae) that are classified within the northern

Supercohort Laurasiatheria, whereas otter shrews have a vague resemblance to the laurasiatherian otters (Carnivora) and many of their semi-aquatic adaptations (Madsen et al. 2001).

There are three species of semi-aquatic otter shrews, one in the genus *Potamogale* and two in the genus *Micropotamogale*. The larger *Potamogale* includes only one species, the Giant Otter Shrew *(Potamogale velox*), and occurs in Central Africa from Western Nigeria to northern Zambia. The smaller *Micropotamogale* includes two species, the Rwenzori Otter Shrew *(M. ruwenzorii*) limited to the Kivu and Ruwenzori regions of the Eastern DR Congo and western Uganda, and the Pygmy or Nimba Otter Shrew (*M. lamottei*) limited to a small area in the Upper Guinea region of West Africa straddling the borders of Liberia, Guinea, and Côte d'Ivoire (Vogel 2013). *Micropotamogale lamottei* is considered "less progressive" than the other two members of the Potamogalinae, based on its brain characteristics (Stephan and Kuhn 1982) and other morphological characters (Guth et al. 1959). The Nimba Otter Shrew was not discovered until 1951 at Ziéla, near Mount Nimba, Guinea (Heim de Balsac 1954). Most of the known records of *M. lamottei* cluster around Mount Nimba, with Sérédou in Guinea to the west and the Putu Mountains in east-central Liberia forming outliers to the main distribution (Figure 1). Kuhn (1964) first noted the presence of *M. lamottei* in the Saniquellie District (Deaple and Kahnple) on the Liberian side of Mount Nimba. Seven years later he reported an additional specimen from the Putu Mountains in east-central Liberia caught on 5 December 1970 in a crab-trap set in a mountain stream "near the camp of the Bong Mining Company near Peloken, Putu Range, Grand Gedeh County, Liberia" (Kuhn 1971, 477), which extended the then known distribution of the Nimba Otter Shrew by about 100 km to the southeast. It was not until almost 40 years later, on 10 November 2010, that three researchers (JD, CRG, JCG) caught another specimen in a minnow trap in a stream probably very close to the 1970 site, in the context of an environmental impact assessment (EIA) for a new iron ore mining project at Putu (Figure 2). The known point localities across the Nimba Otter Shrew distribution result in an extent of occurrence of 57,436 km^2 and an area of occupancy (based on actual habitat suitability) of only 10,851 km^2 (Boitani et al. 2008).

The core habitat of *M. lamottei* around Mount Nimba appears to be in hilly, forested country with many small creeks, swampy areas, and fallow rice fields, where it lives on fresh water crabs, primarily *Liberonautes* spp. (Potamonautidae), but also on ants (Formicidae) and fishes (Figure 3; Kuhn 1964; Vogel 1983). At Putu, the Nimba Otter Shrew was only found in small forest creeks. Ninety percent of all *M. lamottei* captures occurred in water, usually in some kind of fish capture device. Of these, 92 % of individuals were captured in rivers or creeks and only 8 % in swamps (Vogel 1983).

Due to its extremely limited distribution in the Upper Guinea Region of West Africa and various threats from habitat loss resulting from deforestation, farming, and natural resource extraction, the Nimba Otter Shrew has been listed as Endangered with a decreasing population trend on the IUCN Red List (Vogel 2008). It is also included in the list of Evolutionary Distinct and Globally Endangered (EDGE) species. EDGE species are scored according to the amount of unique evolutionary history they represent (Evolutionary Distinctiveness, or ED), and their conservation status (Global Endangerment, or GE; see Isaac et al. 2007 and http://www.edgeofexistence.org). It might be expected that the species also occurs between the main distribution at Mount Nimba and the two disjunct satellite areas of distribution at Sérédou (Ziama) in Guinea, and in the Putu Mountains of Liberia, but to date, evidence for this is lacking. Otter shrews belong to the roughly 70 out of 1,200 living genera of mammals worldwide that are freshwater-dependent, 18 genera (35 species) of which occur in Africa alone. The vast majority of these freshwater mammal species, including all three species of otter shrews, are in IUCN Red List categories higher than Least Concern (Veron et al. 2008), and thus deserving of particular conservation attention. In a phylogenetic and conservation priority analysis, *M. lamottei* ranked highest among Afrotheria for conservation priorities under all criteria used to transform IUCN categories to extinction risk (Kuntner et al. 2011).

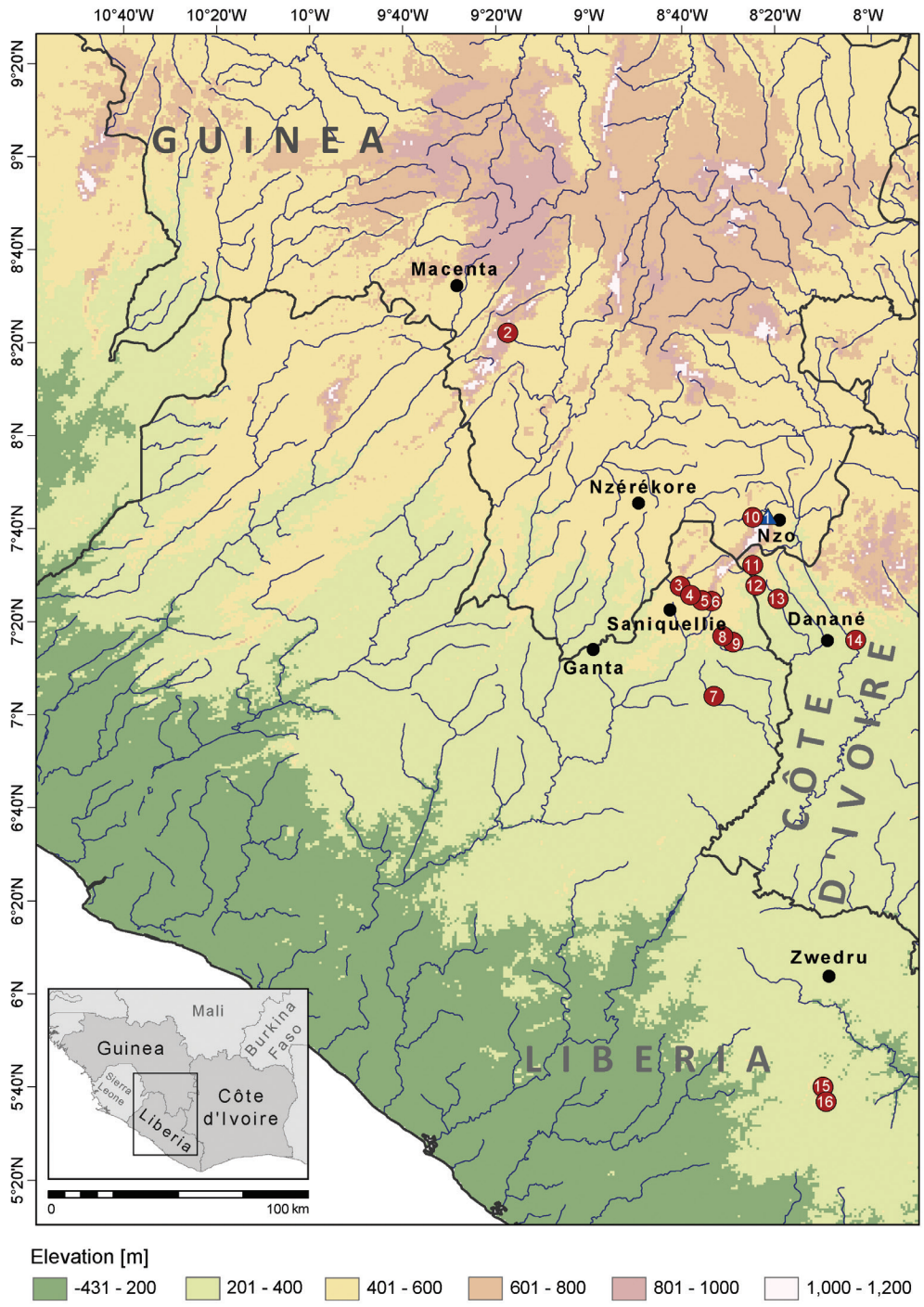


Figure 1. Map of *Micropotamogale lamottei* localities centered on Mount Nimba. Numbered Localities are: 1. Ziéla (= Type locality, alt. 550 m asl, 1951), 2. Sérédou, 3. Tokadeh, 4. Zolowee, 5. East Nimba Nature Reserve (ENNR), 6. Near East Nimba, 7. Deaple, 8. Kahnple, 9, Lapié, 10. Zouguépo, 11. Yéalé, 12. Gbantopleu, 13. Danyapleu, 14. Matonguiné, 15. Slabertsville (Putu), 16. Bong Mining Camp (Putu). Map drawn by Flora Ihlow, ZFMK.



Figure 2. J.C. Garteh holding a minnow trap with a freshly captured *Micropotamogale lamottei* at Putu. The palm nuts (*Elais guineensis*) used to pre-bait the traps to attract freshwater crabs or crayfish are visible within the trap (Photo: © J. Decher 2010).



Figure 3. Nimba Otter Shrew (*Micropotamogale lamottei*) captured at East Nimba and subsequently fitted with a radio-transmitter (Photo: © A. Monadjem).

Micropotamogale lamottei and Human Activities in Its Area of Occurrence - Do Our Assessment Data Matter?

The first detailed description of the Nimba Otter Shrew‘s natural history by Kuhn (1964) already pointed out that this species is affected by local mining. Kuhn (1964) reported that the Gio people had observed the animal falling into the holes of the diamond diggers (dug a few years earlier during the "diamond rush"). Our 2010 survey in the Putu Mountains re-confirmed the occurrence of this species next to a mining camp ("Slabertsville") on the East slope of the Putu Mountains. In fact, during a second survey in 2012, active bulldozing and clearing of forest was occurring adjacent to the stream habitat of *M. lamottei.* We were unsuccessful in verifying the species from elsewhere in or around the Putu Mountains including the northern edge of nearby Sapo National Park (J. Decher, unpublished data).

Unfortunately, relevant biological and ecological data necessary for policy decision-makers and specifically pertaining to the Nimba Otter Shrew are mostly lacking. For example, its total distribution is still incompletely known, probably due to the fact that it is so difficult to capture. Its population density and movement patterns are also poorly known. The population density was estimated as one otter shrew per 10 km^2 by Vogel (2013) based on drowned animals collected by fishermen in fish bownets. We suspect that the figure of one animal per 10 km^2 may have been a print error and that the intended figure may have been one animal per one km^2 , especially since Vogel (1983) tells us that between 1972 and 1976 villagers collected 84 specimens in fish traps just in the relatively small area in Côte d‘Ivoire.

The impact of mining on the Nimba Otter Shrew has already been touched on above. The 1990 IUCN Action Plan (Nicoll et al. 1990) pointed to the devastating mining activities at Mount Nimba as a threat for *M. lamottei* and suggested further research and perhaps a captive breeding program based on the previous successes of keeping the species in captivity (Vogel 1983). Parallel surveys for the Nimba Western Range DSO Iron Ore Project in 2010 found specimens of *M. lamottei* at a proposed Tokadeh stockpile area, which overlaps with a swamp where *M. lamottei* and other globally threatened species occur, and in a small forest stream near Bassa Village less than two km from the East Nimba Nature Reserve (ENNR) (Arcelor Mittal & URS/ Scott Wilson 2010). The local Mah people refer to the otter shrew as "*beng-suno"* (in the Maawe language, *beng* = otter, while *suno* = shrew, so the local and English names have the same meaning; A. Manvell, personal communication). During a recent survey in 2008 of the Guinean side of Mount Nimba, no individuals were located in Ziéla. However, fishermen recognized this species as one that enters in their fish nets. Close to Saniquellie, villagers brought in a specimen and indicated it comes from "bas fond cultivé"(= cultivated depressions; C. Denys, unpublished). In his discussion of the species in the comprehensive *Mammals of Africa*, Vogel (2013) also pointed out that wetland rice agriculture introduced to the Nimba area in 1980 led to large-scale habitat destruction. Since then, more than a dozen individuals have been captured within a 20 km radius of Yekepa, on the Liberian side of Mount Nimba. To date, seven of these individuals have been fitted with radio-transmitters and tracked over several days (A. Monadjem, unpublished data).

Materials and Methods

Otter Shrew Capture Methods

Vogel (1983) succeeded in capturing otter shrews in Sherman traps in Côte d‘Ivoire. At East Nimba Nature Reserve (ENNR), a researcher (BP) deployed three wire mesh funnel traps (= fish traps) and nine Sherman traps with live crab as bait, and captured one otter shrew in a fish trap at Tokadeh (Arcelor Mittal & URS/Scott Wilson 2010). At Putu, the first specimen from 1970 was captured accidentally "in a crab trap that was set in a mountain stream" (Kuhn 1971, 477). Based on this information, our second specimen was captured in 2010 in one of 12 commercially available wire-mesh "Gee Minnow" traps (http://www.memphisnet.net/) placed in the creek beds, usually in deep and quiet pools and under bank overhangs (Figure 4). Two researchers (CD & BK) obtained *M. lamottei* from traditional fishing baskets baited with living crabs quite close to



Figure 4. Small forest creek on the East slope of the Putu Mountains where *Micropotamogale lamottei* was caught (Photo: © J. Decher 2010).

villages and cultivated areas at Zolowee (LB4), Liberia (Figure 5). Likewise, at East Nimba one of us (AM) used similar locally made, funnel-shaped traps. These funnel-traps were placed in shallow streams, side by side from one bank to the next, effectively cutting off the path of an otter shrew traveling in the water.

Molecular Approaches to *Micropotamogale* Population Structure

Genomic DNA from five specimens of *Micropotamogale lamottei* (Table 1) was extracted from liver stored in 95 % ethanol using a modified Gentra Puregene (Qiagen, Valencia, CA) Mouse Tail extraction protocol or NucleoSpin 96 Tissue Kit (Macherey-Nagel, Bethlehem, PA) following the procedure of Vogelstein and Gillespie (1979). The quantity and quality of the extracted DNA was assessed using a NanoDrop 1000 spectrophotometer (NanoDrop, Bethesda, MD). The first 820 bp of the cytochrome *b* mitochondrial gene were amplified by PCR using the primers Cytb-A (L14115) and Cytb-F (H14949) described by Sullivan et al. (1997) and



Figure 5. Typical *Micropotamogale lamottei* habitat in cultivated areas at Zolowee (LB4), Liberia. (Photo: © C. Denys).

Table 1. Percentages of K2P genetic divergence between specimens of the Nimba Otter Shrew (*Micropotamogale lamottei*) from different regions of its area of occurrence in the Upper Guinea Region of West Africa, with the cytochrome *b* divergence in the white and the 12S rRNA divergence in the grey side of the diagonal.

Location			**Yeale**	**Putu**	**Zolowee Nimba**		**East Nimba**	
	Sample		IZEA 5038	ZFMK 2011.0044	LB4	LB149	Ara504	Ara535
		12S rRNA Accession ↓	**Cyt b Accession** →	KU697904	KU697905	KU697906	KU697907	KU697908
Yeale	IZEA 5038	AF390539	----	----	----	----	----	----
Putu	ZFMK 2011.0044	KU726977	0.110	----	0.864	0.616	0.490	0.616
Zolowee Nimba	LB4	KU726978	0.211	0.220	---	0.985	0.864	0.738
	LB149	KU726979	0.000	0.110	0.221	---	0.864	0.738
East Nimba	Ara504	KU726980	0.194	0.098	0.208	0.194	---	0.123
	Ara535	KU726981	0.316	0.331	0.420	0.316	0.000	----

the entire 12S rRNA (957 bp) was amplified with primers 1S (L39) and 3'GW (H1209) described by McNiff and Allard (1998). Double-stranded polymerase chain products were purified with an exonuclease and shrimp alkaline phosphatase digestion. Cycle sequencing was accomplished with dye terminators (ABI, PRISM ver. 3) and excess dye-labeled terminators were removed by Sephadex spin columns. Cycle sequencing products were fractionated and visualized on an Applied Biosystems 373 or 13730XL automated DNA sequencer (Applied Biosystems, ABI, Foster City, CA). Both 12S rRNA and cytochrome *b* sequences were aligned using Clustal X2 (Larkin et al. 2007) and the alignments were further examined by eye. The 12S rRNA sequences obtained were further compared to a sequence from specimen (IZEA 5038) from Yéalé, Cote d'Ivoire obtained from GenBank (Accession # AF390539). PAUP* (Swofford 2002) was used to determine the amount of sequence divergence (uncorrected p) and to calculate Kimura 2-parameter genetic distances (Kimura 1980) between pairwise comparisons of sequences. Arlequin (Excoffier et al. 2005) was used to calculate pairwise F_{ST} between populations.

Results

We captured a total of five (1-Putu; 2-Zolowee; 2-East Nimba) specimens with the various methods described above and then made a first attempt to assess the molecular geographic variation of *Micropotamogale lamottei* throughout its distributional range. Genetic diversity and genetic differentiation was examined from five samples of Nimba Otter Shrews from three localities in Liberia at two mitochondrial loci (cytochrome *b* and 12S rRNA). The 12S sequences were compared with a GenBank sequence (AF390539) from a specimen (IZEA 5038) collected by P. Vogel in Côte d'Ivoire. While all six individuals examined had unique mitochondrial haplotypes, the sequence divergence among these haplotypes was less than 1 %. The number of mutational steps (point mutations) between the composite haplotypes ranged from five to 12 (Figure 6). Estimates of pairwise F_{ST} ranged from 0.00 between Putu and Zolowee to 0.666 between Zolowee and East Nimba and 0.818 between Putu and East Nimba. None of the F_{ST} values were significantly different from zero presumably because of the small sample sizes. Indirect estimates of gene flow provided from these F_{ST} values suggest infinite gene flow between Putu and Zolowee, but only 0.11 and 0.25 migrants per generation between Putu and East Nimba and Zolowee and East Nimba, respectively. However, the unbiased estimates of F_{ST} result in values that are either equal to or less than the true value and thus may overestimate the amount of gene flow.

Although 12S rRNA sequences were incomplete for two of the specimens examined, Putu Specimen JD738 missing the first 50 bases and East Nimba specimen ARA 504 missing data between position 433 and 868, variation was observed at seven positions. Geographic variation and/or polymorphisms observed at these positions of the 957 bases of the 12S rRNA sequences included two insertion-deletions, one of a single base (position 857) and the other of a nine base segment, and five SNPs, four transitions and a transversion. The variations observed at 12 positions among the 820 bases of cytochrome *b* examined in five specimens of Nimba Otter Shrews were all transitions.

The level of genetic divergence (K2P distances) at the cytochrome *b* locus ranged from 0.123 % among specimens from East Nimba to 0.985 % between specimens from Zolowee Nimba (Table 1). The highest divergence was between the two specimens from Zolowee (Nimba), as well as between the one specimen from Zolowee and the specimen from Putu (Table 1). The lowest divergence was between two specimens caught in East Nimba. Genetic divergence was intermediate for other comparisons (Table 1). These cytochrome *b* K2P distances are typical of intraspecific comparisons reported for small mammals.

Less genetic divergence was observed among the 957 bases of the 12S rRNA gene, ranging from zero to 0.42 %, excluding the indels. The greatest genetic differentiation observed was between one of the sequences from Zolowee (LB4) and a sequence from East Nimba (ARA 535), which differed by 4 nucleotide substitutions and a 9 base insertion-deletion. The other sequence obtained from specimens from Zolowee (LB149) was

identical to the 12S rRNA sequence acquired from a specimen from Yéalé (from GenBank). Although one of the sequences procured from East Nimba was incomplete, no differentiation was detected from the two sequences obtained from this area. However, the two sequences from Zolowee differed by two point mutations and a one base insertion-deletion. The greatest genetic differentiation was observed among the specimens from East Nimba and the other three sites sampled with an average K2P genetic distance of about 0.25 % and a nine base insertion-deletion. Mean levels of differentiation were similar among the other three locations (Yeale, Putu, and Zolowee) ranging from a little over 0.1 % to 0.165 %.

Discussion

The difference between the low levels of sequence divergence (K2P) and the high F_{ST} values seen among sites suggests that these three populations were much more contiguous in the past but the East Nimba population is currently receiving relatively little, if any, gene exchange with either the Putu or the Zolowee populations. While the F_{ST} method clearly over-estimates the amount of gene flow, the impact of the small sample sizes on the F_{ST} values is unclear.

Future Direction of Nimba Otter Shrew Research and Conservation

We strongly urge that survey efforts for *M. lamottei* are continued in an increasing radius from Mount Nimba, as well as in the Putu Range and surrounding areas. At Putu, survey sites should include rice fields and low-lying marshy areas. Further sampling at Sapo National Park is necessary to determine the true extent of occurrence of the Nimba Otter Shrew, and to potentially locate additional outlying populations. This would be important for the establishment of offsets for areas that will most likely be lost to mining, while recognizing that offsets within existing protected areas may be insufficient to compensate for such losses (Maron et al. 2015). One researcher (AM) is involved in the implementation of the Nimba Otter Shrew Protection Plan to investigate its ecology, status, and distribution in Liberian Nimba. Methods included two types of locally made funnel traps and also camera traps. However, camera traps were not found to be an efficient means of verifying *M. lamottei* presence (Arcelor Mittal 2013). A three-pronged approach was implemented to gain more information about this elusive species: 1) More extensive habitat surveys were completed in both dry and wet season in all habitats within the

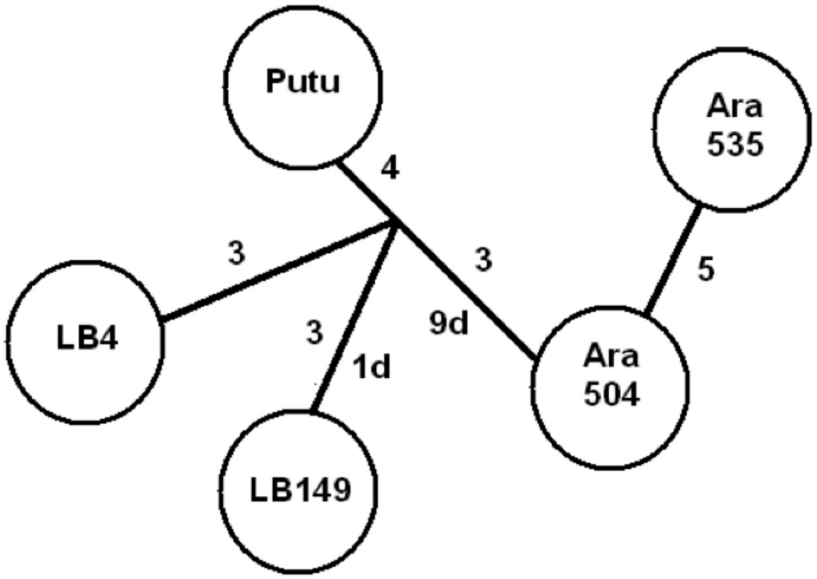


Figure 6. Minimal spanning network of concatenate haplotypes showing the number of point mutations and the one base (1d) and nine base (9d) insertion-deletion along each branch.

species distribution in the Arcelor Mittal mining concession; 2) A pioneering radio-telemetry study was applied that will reveal insights into the spatial ecology and habitat selection of *M. lamottei*; 3) A study was carried out on prey availability and the impact of mining operations on the prey of the Nimba Otter Shrew (mainly crayfish).

At Putu, more traditional trapping efforts using fish traps in the rivers, creeks, and swamps could be combined with an environmental DNA (eDNA) approach using filtered water samples potentially linked to next-generation sequencing (NGS) and eDNA metabarcoding (Goldberg et al. 2011; Taberlet et al. 2012) to assess the wider occurrence of this species in the watershed on the East slope of the Putu Mountains and beyond. A recent critique of the eDNA approach (Roussel et al. 2015) has reinforced the need to combine eDNA detection with other detection methods. By enacting public information campaigns and distributing educational materials to local schools, pride and local awareness of the presence and uniqueness of a globally endangered and extremely restricted species should be raised throughout all known and suspected sites of the distribution of the Nimba Otter Shrew.

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