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Evidence for persistence and a major range extension of the smooth-coated otter (*Lutrogale perspicillata maxwelli*; Mustelidae, Carnivora) in Iraq

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Abstract. The species IUCN conservation status of smooth-coated otter (*Lutrogale perspicillata*) is considered ‘Vulnerable’, due to an inferred future population decline caused by habitat loss and sustained exploitation. The status of the Arabian subspecies (*L. p. maxwelli*) occurring in the Tigris marshes of Iraq and Iran is uncertain due to political problems and limited access to this border region in recent years. With this study we could confirm the persistence of the smooth-coated otter in the marshlands of southern Iraq by using a mitochondrial marker (cytochrome *b*). Moreover, a second sample from Kurdistan was also identified to be *L. perspicillata*. This observation represents a major range extension of more than 500 km for this poorly known species. It is recommended to undertake further surveys of suitable habitat in the Tigris wetlands, as well as in Kurdistan, to obtain additional information on the distribution of smooth-coated otter in Iraq, and implement conservation measures in those areas.

Key words: Middle East, Tigris wetlands, Kurdistan, phylogenetic analysis, mitochondrial marker, conservation status

Introduction

The smooth-coated otter, *Lutrogale perspicillata* (I. Geoffroy Saint-Hilaire, 1826), is an oriental species ranging eastwards from Iraq through the Sind, Nepal and Assam to Indochina, Malaya and Sumatra (Sivasothi & Nor 1994, Hussain et al. 2008, Koepfli et al. 2008, Khan et al. 2010, Lau et al. 2010). The populations of southern Iraq belong to the subspecies *L. p. maxwelli* Hayman, 1956 (Harrison 1968, Harrison

& Bates 1991), and are known to ‘Marsh Arabs’ as the “black otter” (Maxwell 1960). Contrary, Wilson & Reeder (2005) consider the subspecies a synonym of *L. p. sindica* Pocock, 1940. The smooth-coated otter populations of southern Iraq are highly disjunct from the remaining distribution of the species and, therefore, represent an independent demographic unit not connected via gene flow to other populations of south-east Asia. In entire Arabia the subspecies was

only known from two locations in Iraq: Abusakhair, southeast of Amara on the River Tigris (Hayman 1956), and Al Azair village near the Tigris (Hatt 1959). More recently, Ziaee (1996) reported on one skin found in Hawr Al Azim in Iran, and Firouz (2000) included the species in his list of the Iranian fauna, stating that it had been recorded from the marshes close to the Iraqi border in Khuzestan. The smooth-coated otter is essentially a species inhabiting lowlands and plains, adapted to live even in the semiarid regions (Hussain et al. 2008). Generally, it uses large rivers and lakes, peat swamp forests, mangroves and estuaries, and it even uses the rice fields for foraging (Hussain et al. 2008). In Iraq it is restricted to the marshes and wetlands on both sides of the River Tigris.

The current IUCN red list category of *Lutrogale perspicillata* is 'Vulnerable' (A2acd; Hussain et al. 2008), while that of the subspecies *L. p. maxwelli* is reported to be uncertain (Mason & Macdonald 1986). Major threats to Asian populations are the loss of wetland habitats due to the construction of large-scale hydroelectric projects, reclamation of wetlands for settlements and agriculture, reduction in prey biomass, poaching and contamination of waterways by pesticides (Hussain et al. 2008). Since 1977, the smooth-coated otter is listed on Appendix II of CITES. The species is protected in almost all countries where it occurs, but most of them are not able to control the clandestine trade leading to extensive poaching (Hussain et al. 2008). Once common in the wetlands and low lying areas of south Asia, the smooth-coated otter is now restricted to a few protected areas. The creation of corridors between protected areas and the identification of wetlands of national and international importance under the Ramsar Convention has, to some extent, hindered further degradation of suitable habitats for the smooth-coated otter (Hussain et al. 2008). This study was undertaken to document the presence of *L. perspicillata* in Iraq based on cytochrome *b* gene sequences and to report on a large extension of the known range of the species into southern Kurdistan.

Material and Methods

Skins from two otters were collected in the wild in November 2008. The first otter was found dead at Taq Taq area (on the River Nal Zab al Saghir) in Kurdistan, northern Iraq (N 35° 53', E 44° 37'; Fig. 1). The second was purchased from a hunter who killed the animal on the Iraqi side of the Hawizeh Marshes (N 31° 37', E 47° 43'; Fig. 1). Based on morphological and phenotypic characters (i.e., dark, smooth and

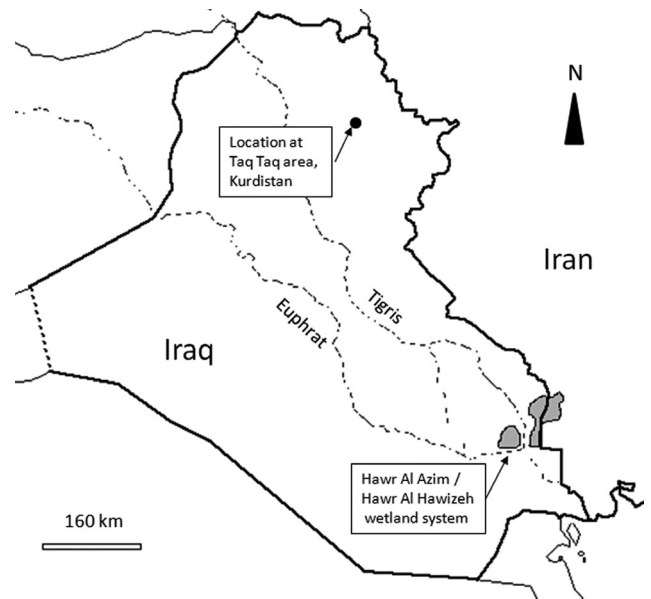


Fig. 1. Location of the Hawr Al Azim/Hawr Al Hawizeh wetland system on both sides of the Iraqi-Iranian border and the location of the River Nal Zab al Saghir, Taq Taq area in Kurdistan.

sleek pelage, dorso-ventrally flattened tail, rhinarium with sharply defined hair-line), both specimens were assumed to belong to the smooth-coated otter, meaning that the specimen collected from Kurdistan represents a record more than 500 km northbound of the closest known population. In order to confirm this morphological classification, sequences of the mitochondrial cytochrome *b* gene were obtained from both skin samples and compared phylogenetically with those available from 11 other otter species.

DNA was extracted using the DNeasy blood and tissue kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions. The complete coding region of the cytochrome *b* gene was amplified (i.e. ~1140 bp) using the versatile primers L14724 and H15915 (Kocher et al. 1989, Irwin et al. 1991). All reactions were performed in a 25 µl reaction volume with 1 µl of the sample DNA, 0.5 units of *Taq* polymerase (Bioline, UK), 1.5 mM MgCl₂, 40 µM of each dNTP and 200 nM of each primer. Amplifications were performed with an initial denaturation of 2 min at 92 °C, followed by 35 cycles of 92 °C for 30 seconds, 50 °C for 30 seconds and 72 °C for 30 seconds and a final extension (72 °C for 5 minutes).

PCR products were checked on a 1.4 % agarose gel. Successfully amplified PCR products were purified using a gel purification kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions. Sequencing was conducted with the same PCR primers as used for PCR amplification, using an

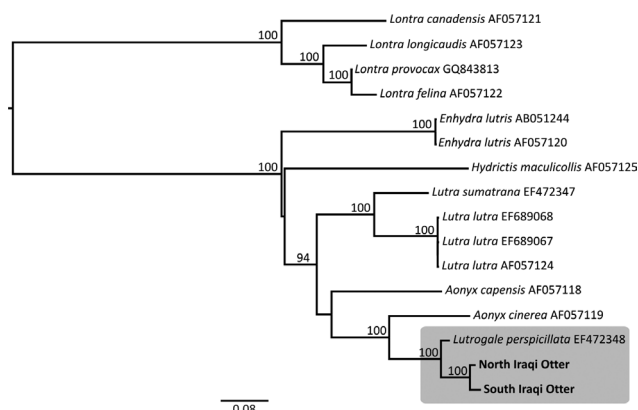


Fig. 2. Maximum Likelihood (ML) tree of the cytochrome *b*-dataset using the GTR + Γ + I model. Maximum Likelihood bootstrap-support values for the best-scoring ML tree were estimated from 100 bootstrap replicates given at the branches. For sequences of other otter species obtained from GenBank, the accession numbers are provided next to the species names. The parameters for the GTR + Γ + I model of nucleotide substitutions are as follows: Γ shape parameter $\alpha = 11.6555$, $I = 0.5902$ with $a - \ln L = 4624.2035$.

automated laser fluorescent DNA sequencer (ALF) and dye-terminator chemistry (Pharmacia Biotech). Both sequences have been submitted to GenBank (accession numbers JQ437613 and JQ437614).

The obtained cytochrome *b* sequences of Iraqi otters were aligned with Genbank sequences (accession numbers given in Fig. 1) from *Lutrogale perspicillata* and other otter species (*Aonyx cinerea*, *A. capensis*, *Lutra lutra*, *L. sumatrana*, *Hydrictis maculicollis*, *Enhydra lutris*, *Lontra canadensis*, *L. longicaudis*, *L. felina* and *L. provocax*) using the alignment implementation built in to the software Geneious Pro v5.1.7 (Drummond et al. 2010). The phylogeny of the alignment was inferred by a Maximum Likelihood analysis (ML) using the web-based version of RA \times ML 7.2.8 (Stamatakis 2008), using GTR + Γ + I model parameters. GTR is the only available nucleotide substitution model in RA \times ML. River otter species of the new world (genus *Lontra*) were used as outgroup due to their sistergroup relationship to the old world river otter clade (*sensu* Koepfli et al. 2008). The nodal support values for the best-scoring ML tree were inferred from 100 bootstrap replicates. In order to evaluate the findings of our ML analysis we further performed Neighbor-Joining (NJ) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithms

with the substitution models Jukes-Cantor, HKY and Tamura-Nei, respectively, implemented in to the software Geneious Pro v5.1.7 (Drummond et al. 2010). The nodal support values for these analyses were inferred from 2000 bootstrap replicates.

Results

Both samples amplified successfully and in the case of the sample from Kurdistan the complete cytochrome *b* sequence could be obtained. For the otter sampled at Hawizeh Marshes we could only obtain 693 nucleotides of the cytochrome *b* gene. Phylogenetic analysis using ML revealed high nodal support for the two otter specimens belonging to the smooth-coated otter (*Lutrogale perspicillata*) and confirmed the morphological and phenotypic classification of the skins (Fig. 2). The NJ and UPGMA analyses with the different substitution models also supported the assignment of the two unknown otters to *Lutrogale perspicillata* with a nodal bootstrap support of 100 in any case.

Furthermore, both specimens represent distinct haplotypes that differ from the reference sequence with 30 variable sites (2.7 %) for the otter originated from Kurdistan and 19 variable sites (2.8 %) for the otter originated from Hawizeh Marshes, respectively, as well as from each other in seven nucleotides (1 %) with high bootstrap support (Fig. 2). Concerning the insufficient molecular data throughout the distribution range of the species, any suggestions regarding the subspecies taxonomy or phylogeography of smooth-coated otter would be highly speculative.

Discussion

Our data confirm that both otter skins collected in Iraq can be assigned to *Lutrogale perspicillata* (Fig. 2), and, therefore, confirm the continued persistence of the smooth-coated otter in the Hawizeh Marshes of southern Iraq for the first time since the published observations of Hayman (1956), Hatt (1959) and Maxwell (1960). Furthermore, our results represent a major extension of the species' range, reaching as far north as the Arbil Province in Kurdistan, northern Iraq. This new location extends the distribution of smooth-coated otter by more than 500 km to the north-west.

Given the extent of habitat loss that is occurring in south and south-east Asia and the intensity of poaching, a serious reduction in population size has been observed in many parts of the species' range (Hussain 1993, Melisch et al. 1996, Hussain & Choudhury 1997, Hussain 2002). Although quantitative data on

population sizes or trends are lacking, it is suspected that the global population of the smooth-coated otter has declined by > 30 % over the past 30 years (Hussain et al. 2008). Concerning the difficult political situation Iraq has faced in the last decade, no information on status and distribution of smooth-coated otter in Iraq is currently available. The potential impact of oil exploration on wetland habitats and political turmoil in the region are major concerns for conservation, and consequently the survival of smooth-coated (and possibly Eurasian) otters in the region. For example, the Taq Taq region and the areas of the Hawizeh Marshes contain major oilfields and are subject to further development, making implications for otter conservation even more urgent.

On the Iranian side of the Tigris (Hawr al Azim wetlands), current knowledge on the distribution and status of the species is also limited, and attempts to obtain more information were often hampered by military constraints. A recent survey (Mirzaei et al. 2010) found no signs of otters in the area, although a considerable amount of time was spent searching the water stretches during different seasons. However, fishermen that were interviewed by the authors claimed that otters were sometimes observed in the inner or

upper parts of Hawr Al Azim, but they were unable to distinguish between smooth-coated otter and the Eurasian otter (*Lutra lutra*). Therefore, it remains still unclear whether smooth-coated otters currently exist in the Iranian part of the Tigris marshes.

Still, our findings give reason for hope that the smooth-coated otter is, beside the Eurasian otter, still occurring in the Tigris wetlands and marshes on both sides of the Iraqi border. However, it is imperative to further survey suitable habitats in the Euphrates/Tigris basin in order to obtain additional information on the distribution and status of *Lutrogale perspicillata* in Iraq and Iran. Moreover, it is necessary to confirm the existence of smooth-coated otter in Kurdistan, to identify locations and habitats in this new part of the species' range, and to implement conservation measures in those areas.

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