NOTE

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Molecular markers for some small mammals of southern Africa

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A b s t r a c t . Primers to amplify partially the mitochondrial genes coding for cytochrome b, 12S-rRNA and the D-loop were screened in twelve small mammal species from southern Africa. We also tested the possibilities of molecular sex identification using primers of the SRY. The study includes five species of murids of the genera *Aethomys*, *Thallomys*, *Rhabdomys* and *Saccostomus*, four gerbils of the genera *Desmodillus*, *Tatera*, *Gerbillurus*, one dormouse (*Graphiurus*) and two macroscelids (*Macroscelides* and *Elephantulus*). We provide information on the primers with modifications, lab protocols and we give an overview of the obtained sequences: four cytochrome b sequences and five 12S-rDNA sequences of three species and 23 D-loop sequences of five species. Sex specific fragments of SRY could be amplified in three species of Murinae and the dormouse.

Key words: sex identification, SRY, mtDNA, rodents, macroscelids, Kalahari

Introduction

An increasing number of studies use small mammals to monitor environmental changes. Beside data on abundance and structure of small mammal populations, the consequences of habitat loss and fragmentation for genetic structure within and gene flow among populations became a focus in conservation biology. In southern Africa the rapidly increasing intensity of land use in savannahs calls for easy-to-use and reliable molecular markers to evaluate for small mammals the consequences of land use on genetic structure. Furthermore, despite numerous studies on the ecology of small mammals of southern African, there is still considerable debate and confusion on their taxonomy and phylogeny (C h e v r e t & D o b i g n y 2005). Museum collections provide important sources to evaluate such problems. Within this note we tested primers to amplify a part of the mitochondrial cytochrome b and 12S-rRNA gene as well as the D-loop in twelve species of small mammals occurring across southern Africa. We also included museum material. Many specimens in museums are not sexed. Therefore we additionally tested two marker sets for a sex-specific region of the SRY gene (P o m p et al. 1995, S á n c h e z et al. 1996).

Material and Methods

Tissue samples (10–15 mm^2 piece of the ear) were obtained from live-trapped animals and stored in 96% ethanol. DNA was extracted using spin columns (DNeasy-tissue kit; Qiagen, Hilden, Germany; yields 30 to 100 ng / µl). Additional DNA was extracted from museum specimen of the black-tailed tree rat *Thallomys nigricauda* and the acacia rat *Thallomys paedulcus*

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(K o h n e n 2006, nomenclature of common names follows B r o n n e r et al. 2003). It was necessary to remove the hairs from all samples of *Thallomys* with a scalpel as hairs inhibited amplifications. Other chemicals (e.g. borax) in skin samples were removed by dialysis (Millipore "V" series membranes, Molsheim, France; 0.025 μm pore width). PCR protocols were optimised for each marker system by testing several temperatures (range tested: 50–65°C), time and concentrations until we obtained reliable results (Table 1).

Table 1. PCR protocols for amplifying sex-specific DNA-fragments and mitochondrial DNA (partial, see text). All protocols have an extension step of 1 minute at 72°C in each cycle and a final extension of 5 minutes at 72°C. Each PCR reaction was carried out with 1 unit Taq DNA-polymerase (recombinant) in 10 mM Tris-HCl-buffer (pH 8.3) and 50 mM KCl (all SIGMA, Taufkirchen, Germany). The amount of template DNA was 10 – 20 ng for sex identification and 20 – 200 ng for amplification of mitochondrial DNA.

	Mitochondrial DNA			Sex identification	
	Cytochrome b	12S-rRNA	D-loop	P o m p et al. (1995)	S á n c h e z et al. (1996)
Initial	94°C / 4 min	94°C / 3 min	94°C / 4 min	94°C / 4 min	93°C / 5 min
Number of cycles	32	35	32	32	30
Denaturation	94°C / 30 s	94°C / 1 min	94°C / 30 s	94°C / 30 s	93°C / 1 min
Annealing	52°C / 30 s	58°C / 1 min	62°C / 30 s	50°C / 30 s	50°C / 1 min
$MgCl_2$	1.5 mM	2 mM	1.5 mM	1.5 – 4 mM	1.5 – 4 mM
dNTP each	20 μM	50 μM	20 μM	50 μM	50 μM
Primer forward + reverse each	0.5 μM	0.4 μM	0.5 μM	0.5 μM	0.2 μM
Internal control	-	-	-	0.05 μM	0.1 μM

PCR products were run on 2% agarose gels (5 μl of products), stained with ethidium bromide (2 mg/l, 20 min) and visualised under UV. The products were purified using the Qiagen MinElute PCR purification kit (Qiagen, Hilden, Germany). Sequencing was performed by Sequencing Laboratories Göttingen GmbH (Göttingen, Germany). Sequence data were edited using BioEdit version 7.0.5.2 (H a l l 1999) and judged by the Basic Local Alignment Search Tool of the NCBI database. In case of insufficient quality of sequences obtained, primers were modified for better results. The reliability of protocols was checked by repeating amplification and sequencing. For the present note we sequenced only products for species with no entry for the respective gene in GenBank.

We also checked primer pairs for sex identification in the twelve species (P o m p et al. 1995; SRY-A5, 5´-TGA ACG CAT TCA TGG TGT GGT-3´ and SRY-A3; 5´-AAT CTC TGT GCC TCC TGG AA-3; fragment length of 157 bp) and (S á n c h e z et al. 1996; 5´-GTC AAG CGC CCC ATG AAT GCA T and 5´-AGT TTG GGT ATT TCT CTC TGT G-3´; 202 bp). For each species primers were tested in two females and two males. SRY primers were not screened in *T. paedulcus* as only museum material was available. This material was not sexed and we had no reliable information to cross-check the molecular results. The primer for the 12S-rRNA (450 bp) served as an internal control.

Results and Discussion

During our tests we sequenced four new partial sequences of cytochrome b (length 547–763 bp), five of 12S-rRNA (378–414 bp) and 23 of the D-loop region (290–490 bp; Table 2).

For the two macroscelids (round-eared elephant-shrew *Macroscelides proboscideus* and bushveld elephant-shrew *Elephantulus intufi*) we obtained no PCR products. Our protocols failed also in the namaqua rock mouse *Aethomys namaquensis*, the hairy-footed gerbil *Gerbillurus paeba* and the brush-tailed hairy-footed gerbil *G. vallinus*. For all other species, the cytochrome b-fragment was amplified combining the primers L14723 (5´-ACC AAT GAC ATG AAA AAT CAT CGT T-3´; Ducroz et al. 2001) and H15553 (5´-TAG GCA AAT AGG AAA TAT CAT-3´; modified from Ducroz et al. 2001, Colangelo et al. 2005). The 12S-rRNA gene fragment was amplified combining the primers L1091; (5´-AAA CTG GGA TTA GAT ACC CCA CTA T-3´) and H1478 (5´-GAG GGT GAC GGG CGG TGT GT-3´; modified from Kocher et al. 1989).

Table 2. Results of screening primers of fragments of mitochondrial genes (t.n.s. = tested but not successful) and for the sex-specific SRY gene (Yes = reliable amplification; No = no products; n.t. = not tested) in some small southern African rodents. Accession numbers (NCBI, GenBank) are given for sequences resulting from this study. See text for species screened but with no successful amplification.

(sub-)family	Species	Mitochondrial DNA			Sex identification	
		Cytochrome b	12S-rRNA	D-loop	Pomp et al. (1995)	Sánchez et al. (1996)
Murinae						
	Thallomys nigricauda	DQ381925	DQ381930	DQ381935 to DQ381950	Yes	Yes
	Thallomys paedulcus	DQ381926 DQ381927	DQ381931 DQ381934	n.t.	n.t.	n.t.
	Saccostomus campestris	t.n.s.	DQ652189 DQ652190	DQ652187 DQ652188	Yes	Yes
	Rhabdomys pumilio			DQ652186	Yes	Yes
Gerbillinae						
	Tatera leucogaster			DQ652191 DQ652192	No	No
	Desmodillus auricularis			DQ652183 DQ652184	No	No
Gliridae						
	Graphiurus parvus	DQ652185		t.n.s.	Yes	No

The primers of the D-loop varied among the species investigated. For the genus *Thallomys* new primers had to be designed (L16007; 5´-CAG CAC CCA AAG CTG GTA TT-3´ and H16393; 5´-TTG TTG GTT TCA CGG AGG AT-3´, Kohnen 2006). The primer pair L15925 (5´-TAC ACT GGT CTT GTA AAC C-3´) and H16499 (5´-CTT GAA GTA GGA ACC AGA T-3´; accession no. AY576877) was used in the pouched mouse *Saccostomus campestris*, the bushveld gerbil *Tatera leucogaster* and the cape short-tailed gerbil *Desmodillus auricularis*. For the four-striped grass mouse *Rhabdomys pumilio* the primer pair L15997 (5´-TCC CCA TCA GCA CCC AAA GC-3´) and H16401 (5´-TGG GCG GGT TGT TGG TTT CAC GG-3´; Stacy et al. 1997) resulted in high quality amplifications.

For the three species of the subfamily Murinae the molecular sex identification matches in all cases the morphological sex identification. In the savannah dormouse *Graphiurus parvus* only the primers described by P o m p et al. (1995) amplified the sex-specific product. In the other species the tested SRY primers produced no product. Already B r y j a & K o n e č n y (2003) noted that the applicability of the SRY markers might vary even between closely related species.

A c k n o w l e d g m e n t s

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