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Limited efficiency of universal mini-barcode primers for DNA amplification from desert reptiles, birds and mammals

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ABSTRACT. In recent years, DNA barcoding has emerged as a powerful tool for species identification. We report an extended validation of a universal DNA mini-barcode for amplification of 130-bp COI segments from 23 specimens collected from a desert environment, including 11 reptiles, five mammals and seven birds. Besides the standard double-annealing protocol, we also tested a more stringent single-annealing protocol. The PCR success rate for the amplification of the mini-barcode region was: mammals (4/5), reptiles (5/11) and birds (4/7). These findings demonstrate the limited utility of universal primers for mini-barcoding, at least for these vertebrate taxa that we collected from the Saudi Arabian desert.

Key words: Mini-barcode; Cytochrome oxidase 1; Reptiles; Mammals; Birds; PCR; Barcoding; Amplification success

INTRODUCTION

DNA barcoding has become a promising tool for the rapid and accurate identification of various species (Hebert et al., 2003a; Dawnay et al., 2007). Animal DNA barcodes (600- to 800-bp segments) of the mitochondrial cytochrome oxidase I (COI) gene have been proposed as a means for use in species identification, phylogenetics and biodiversity analysis (Hebert et al., 2003b; Valentini et al., 2009; Khan et al., 2010). Although the beginning portion of the COI gene (COI-1) constitutes a basis of a standard DNA barcode due to availability of conserved primers for numerous taxa, the middle (COI-2) and terminal (COI-3) regions have also been found to be useful for the identification of diverse taxonomic classes (Ivanova et al., 2007; Clare et al., 2007). Lorenz et al. (2005) have suggested that depositing barcode sequences in a public database, along with primer sequences, trace files and associated quality scores, would make this technique widely accessible for species identification and biodiversity analysis.

Recently, Meusnier et al. (2008) have reported a universal DNA mini-barcode for biodiversity analysis, with particular application for archival specimens or those with degraded DNA. The 130-bp mini-barcode appears to be more efficient than the standard 650- bp barcode for diverse taxonomic groups including mammals, fishes, birds and insects (Meusnier et al., 2008). Thus, to widen the scope of this mini-barcode, we evaluated the validity of relevant universal primers (Uni-MinibarF1 and Uni-MinibarR1) for the identification of various reptiles, mammals and birds of a desert environment.

MATERIAL AND METHODS

This study was conducted on 23 specimens, including 11 reptiles, 5 mammals and 7 birds (Table 1). Part of the sample collection was conducted with the support of the National Commission for Wildlife Conservation and Development and National Wildlife Research Center, Taif, Saudi Arabia. The DNA was extracted from blood (reptiles and mammals) or tissue (birds) samples using the DNeasy Blood and Tissue kit (Qiagen GmbH, Germany) with the automation of the QiaCube robotic platform (Qiagen), according to manufacturer instructions. The extracted DNA was finally dissolved in 200 µL elution buffer and stored at -20°C.

The mini-barcode (130 bp) was amplified using the primer pair Uni-MinibarF1 (TCC ACT AAT CAC AAR GAT ATT GGT AC) and Uni-MinibarR1 (GAA AAT CAT AAT GAA GGC ATG AGC), as reported earlier (Meusnier et al., 2008). The reactants contained 12 µL FideliTaq PCR master mix (USB Corporation, USA), template DNA (2 µL), each primer (50 nmol in 0.5 µL) in a reaction volume of 25 µL. Besides the standard protocol based on two sequential annealing temperatures (Meusnier et al 2008), we also tested the PCR amplifications using a more stringent (single-annealing) protocol. The thermal cycling conditions for both these protocols are summarized in Table 2. The PCR products were electrophoresed on a 1% agarose gel stained with ethidium bromide. The bands in the gel were visualized under UV light and evaluated using a Proxima C16 Phi+ gel imaging system (Isogen Life Science, The Netherlands).

RESULTS AND DISCUSSION

The results of this study clearly demonstrated the limited performance of universal mini-barcode primers Uni-MinibarF1 and Uni-MinibarR1 for amplification of the specific

Table 1. Details of samples tested for mini-barcoding.

SN	Species	Common Name	Class	Order	Family
1	*Acanthodactylus schmidti*	Schmidt's fringe-toed lizard	Reptilia	Squamata	Lacertidae
2	*Acanthodactylus schmidti*	Schmidt's fringe-toed lizard	Reptilia	Squamata	Lacertidae
3	*Diplometopon zarudnyi*	Zarudnyi's worm lizard	Reptilia	Squamata	Trogonophidae
4	*Ptyodactylus hasselquistii*	Fan-footed gecko	Reptilia	Squamata	Gekkonidae
5	*Stenodactylus doriae*	Arabian whip tailed gecko	Reptilia	Squamata	Gekkonidae
6	*Scincus scincus*	Sand fish	Reptilia	Squamata	Scincidae
7	*Scincus scincus*	Sand fish	Reptilia	Squamata	Scincidae
8	*Scincus mitranus*	Eastern sand fish	Reptilia	Squamata	Scincidae
9	*Scincus mitranus*	Eastern sand fish	Reptilia	Squamata	Scincidae
10	*Uromastyx microlepis*	Spiny-tailed lizard	Reptilia	Squamata	Agamidae
11	*Uromastyx microlepis*	Spiny-tailed lizard	Reptilia	Squamata	Agamidae
12	*Oryx leucoryx*	Arabian oryx	Mammalia	Artiodactyla	Bovidae
13	*Acinonyx jubatus*	Cheetah	Mammalia	Carnivora	Felidae
14	*Panthera pardus nimr*	Arabian leopard	Mammalia	Carnivora	Felidae
15	*Hyaena hyaena*	Striped hyena	Mammalia	Carnivora	Hyaenidae
16	*Hyaena hyaena*	Striped hyena	Mammalia	Carnivora	Hyaenidae
17	*Alectoris melanocephala*	Arabian partridge	Aves	Galliformes	Phasianidae
18	*Alectoris melanocephala*	Arabian partridge	Aves	Galliformes	Phasianidae
19	*Streptopelia senegalensis*	Laughing dove	Aves	Columbiformes	Columbidae
20	*Passer domesticus*	House sparrow	Aves	Passeriformes	Passeridae
21	*Merops orientalis*	Green bee eater	Aves	Coraciiformes	Meropidae
22	*Torgos tracheliotus*	Lappet-faced vulture	Aves	Falconiformes	Accipitridae
23	*Torgos tracheliotus*	Lappet-faced vulture	Aves	Falconiformes	Accipitridae

SN = specimen number.

Table 2. Thermal cycling conditions for PCR amplifications.

Protocol	Stage 1	Stage 2	Stage 3	Stage 4
Double annealing	95 (2) × 1	95 (1) → 46 (1) → 72 (0.5) × 5 95 (1) → 53 (1) → 72 (0.5) × 35	72 (5)	4 (∞)
Single annealing	95 (2) × 1	95 (1) → 53 (1) → 72 (0.5) × 40	72 (5)	4 (∞)

The parameters for each stage are: temperature, °C (time, min) × number of cycles. Stage 4 is the end of PCR.

COI gene segment, especially from reptiles and birds. Ten samples showed positive PCR amplification using single-annealing protocol, whereas only 8 samples showed specific bands with the double-annealing protocol (Figure 1). Taking into account the cumulative performance, this mini-barcode resulted in a comparatively higher PCR success in mammals (80%) than reptiles (45.5%) and birds (57.1%) (Table 3). The DNA samples used in this study were obtained from blood or tissues and were free of any type of degradation. However, the limited success of the mini-barcode universal primers for our specimens is not surprising. Elias et al. (2007) have also noticed the poor performance of DNA barcoding, with only 77% unambiguous identification of species using a standard 653-bp arthropod barcode. The most relevant cause of the failed amplifications for COI mini-barcode can be a primer mismatch at the complementary annealing site. Actually, in certain taxonomic groups, there could be enough variation in the flanking segments on either side of the barcode to require multiple primer combinations instead of a couple of universal primers for gaining amplicon success. Thus, several investigators have tended to rely on species-specific barcodes for greater specificity and sensitivity (Ward et al., 2005; Dubey et al., 2011).

The positive amplification for specimens 8, 14, 15, 22 and 23 using the single-annealing protocol (Table 3) may have been due to the use of perfect annealing temperatures for all 40 cycles of PCR. On the other hand, a large number of nonspecific/multiple bands in

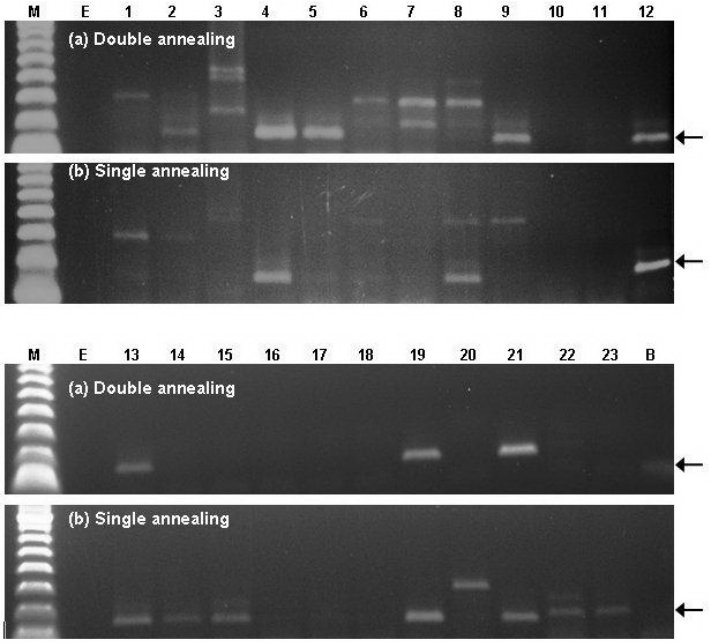

Figure 1. Agarose gel electrophoretogram. Arrows indicate the minibar-specific band. *Lane M* = size marker (100-bp ladder); *lane E* = empty lane; *lane B* = reagent blank; *lanes 1-23* = specimen numbers.

Table 3. Mini-barcode amplification success for various species.

SN	Species	Annealing		Class (AS)
		Single	Double	
1	Acanthodactylus schmidti	×	×	Reptilia (45.5 %)
2	Acanthodactylus schmidti	×	✓	
3	Diplometopon zarudnyi	×	×	
4	Ptyodactylus hasselquistii	✓	✓	
5	Stenodactylus doriae	×	✓	
6	Scincus scincus	×	×	
7	Scincus scincus	×	×	
8	Scincus mitranus	✓	×	
9	Scincus mitranus	×	✓	
10	Uromastyx microlepis	×	×	
11	Uromastyx microlepis	×	×	
12	Oryx leucoryx	✓	✓	Mammalia (80.0 %)
13	Acinonyx jubatus	✓	✓	
14	Panthera pardus nimr	✓	×	
15	Hyaena hyaena	✓	×	
16	Hyaena hyaena	×	×	
17	Alectoris melanocephala	×	×	Aves (57.1 %)
18	Alectoris melanocephala	×	×	
19	Streptopelia senegalensis	✓	✓	
20	Passer domesticus	×	×	
21	Merops orientalis	✓	✓	
22	Torgos tracheliotus	✓	×	
23	Torgos tracheliotus	✓	×	

✓ and × indicate success and failure, respectively. SN = specimen number; AS = amplification success.

the double-annealing protocol (Figure 1) could have resulted from the use of a low annealing temperature (46°C) during the first 5 cycles. The other observation of nonspecific bands could have arisen from the use of a degenerate forward primer (R at location 15). The problematic co-amplification of nonfunctional copies of mtDNA from nucleus, the so-called nuclear mitochondrial pseudogenes (numts), with orthologous mtDNA by using conserved universal primers may also occur in certain cases. Song et al. (2008) have demonstrated that the presence of COI numts can introduce serious ambiguity into DNA barcoding by overestimating the number of species. Moreover, the co-existence of more than one type of mtDNA genome (sequence variation due to substitutions or insertions/deletions) in the same individual (heteroplasmy) is yet another challenge to the barcoding approach (Rubinoff et al., 2006). Although the cases of failed PCR amplifications may be resolved by employing degenerate primers (Sorenson et al., 1999), their use tends to increase the chance of co-amplification of other regions (Zhang and Hewitt, 1996) as well as numts (Lorenz et al., 2005).

In conclusion, despite the reported efficiency of the universal mini-barcode primers Uni-MinibarF1 and Uni-MinibarR1 for diverse taxonomic groups, they appeared to be of limited value for most of the species tested in our study, particularly reptiles and birds of the desert environment.

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