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Original investigation

Javan mongoose or small Indian mongoose–who is where?

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ABSTRACT

The Javan mongoose *Urva javanica* and the small Indian mongoose *Urva auropunctata* have been recently shown not to be conspecific. However, the limits of their respective distribution ranges have not been fully defined. In particular, Chinese populations were not attributed to either species using molecular data. Furthermore, the small mongooses found on Hong Kong Island (discovered at the end of the 1980s) were not clearly attributed to *U. auropunctata* or *U. javanica*, nor their status (native or introduced) established. The main aims of this study were to: (1) investigate the intraspecific genetic diversity and structure within these two species; and (2) clarify the distribution limits of *U. auropunctata* and *U. javanica*, and in particular, to identify Chinese populations, and determine which species occurs on Hong Kong Island (and whether they are native or introduced). The analyses of one nuclear and three mitochondrial genes confirmed the separation of *U. javanica* and *U. auropunctata*, and showed that the populations from southern China and Hong Kong Island belong to *U. javanica*. The intraspecific geographical structure of the two species is clarified, and the taxonomic implications are discussed. In particular, we found a strong divergence of Javan individuals of *U. javanica*, which should be considered a separate subspecies.

Introduction

Nine species of mongoose occur in Asia (Patou et al., 2009a; Gilchrist et al., 2009), and recent molecular studies have shown that they form a monophyletic group that should be placed in the genus *Urva* (Patou et al., 2009a; Egi et al., 2011; Veron et al., 2015). The Javan mongoose *Urva javanica* (É. Geoffroy Saint-Hilaire, 1818) occurs in Southeast Asia, while the small Indian mongoose *Urva auropunctata* (Hodgson, 1836) is found from the Arabian Peninsula across the northern Indian subcontinent to Southeast Asia (Gilchrist et al., 2009; Jennings and Veron, 2011). The latter was introduced at the end of the 19th century and during the 20th century to many different parts of the world (mainly on islands) for biological control of rats and snakes in plantations (Tvrtkovic and Kryštufek, 1990; Simberloff et al., 2000; Thulin et al., 2006; Barun et al., 2013); it is considered by the IUCN to be among the "100 of the world's worst invasive alien species" (Lowe et al., 2000).

These mongooses were considered either as two species, or as one single species (usually named *Herpestes javanicus*, see Wozencraft, 2005 and Veron et al., 2007), varying in size and colour over its range (Simberloff et al., 2000). Veron et al. (2007), using mitochondrial DNA, found three distinct clades corresponding to the Javan mongoose, the small Indian mongoose, and the Indian grey mongoose *Urva edwardsii* (É. Geoffroy Saint-Hilaire, 1818), with a mean Cytb genetic divergence of 5% between each pair of species. The mitochondrial analyses of Veron et al. (2007) supported a sister relationship between *U. javanica* and *U. edwardsii*, whereas Patou et al. (2009a), using nuclear data, found that *U. javanica* and *U. auropunctata* were sister species (although the node supports in the phylogenetic trees were low).

The genetic separation of *U. javanica* and *U. auropunctata* into two species is supported by the morphometric study of Taylor and Matheson (1999); studies of coat colour variation also concur with these results. *U. javanica* specimens from Vietnam and Java are reddish, while *U. auropunctata* specimens from northwest India and Pakistan are paler, and those from Assam and Myanmar are darker and greyish (Pocock, 1941; Corbet and Hill, 1991). This is in agreement with our personal observation of 341 specimens from nine museums (see acknowledgements); those from Thailand, Laos and Java (*U. javanica*) are dark brown and reddish (particularly on the head), while those from India, Nepal and Pakistan (*U. auropunctata*) are paler or greyish.

Even though there is genetic and morphological evidence for the separation of the Javan mongoose and small Indian mongoose into

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two species, very little is known about their intraspecific genetic diversity and geographical structure, and their precise distribution limits. Defining the distribution limits and the intraspecific genetic diversity of these two mongooses is needed for assessing the taxonomic and conservation status of these poorly studied species. Three subspecies of *U. javanica* and five subspecies of *U. auropunctata* have been mentioned by Gilchrist et al. (2009), but their validity has not been tested using molecular data. Chinese populations were previously assigned to the small Indian mongoose (Ellerman and Morrison-Scott, 1951; von Michaelis, 1972; Honacki et al., 1982; Taylor and Matheson, 1999), and mongooses from Hainan and southern China were grouped in the subspecies *U. auropunctata rubrifrons* (J. Allen, 1909) by Ellerman and Morrison-Scott (1951), but these assertions have not been tested using molecular data. Furthermore, the identity and status of mongooses on Hong Kong Island were unclear. The first record of a small mongoose on Hong Kong seems to be an individual trapped at Mai Po in 1989 (Corlett, 2001), but the identity and status (native or introduced) of this species were uncertain, and it now seems to have largely spread across Hong Kong (Shek et al., 2007; Lau et al., 2010).

The main aims of this study were to: (1) investigate the intraspecific genetic diversity and geographical structure within *U. javanica* and *U. auropunctata*; and (2) clarify the distribution limits of the Javan and small Indian mongooses, and in particular, to assign Chinese populations to one of these two species, and to test which species occurs on Hong Kong Island (and whether native or introduced). For this purpose, we sequenced three mitochondrial fragments, Cytochrome b (Cytb), Control Region (CR) and NADH dehydrogenase subunit 2 (ND2), and one nuclear gene, Beta-fibrinogen intron 7 (FGB).

Material and methods

Both fresh (hairs or tissue) and museum samples were used in this study (Table 1, Fig. 1). Sequences of a total of 47 individuals of *U. auropunctata*, *U. edwardsii* and *U. javanica* were analysed (from this study, previous studies, and GenBank; see Table 1). We used *U. brachyura* and *U. fusca* as outgroups, following the results of Patou et al. (2009a).

Total genomic DNA was isolated from samples following a CTAB-based protocol (Winnepenninckx et al., 1993). For museum specimen samples, Dithiothreitol (DTT; 1 M) was added during the tissue lyses and the digestion time was increased (up to 72 h). We used Cytb primers from Veron et al (2004a,b), ND2 primers from Perez et al. (2006) and Patou et al. (2009a), and CR primers as in Patou et al. (2009b). The nuclear locus FGB was amplified using primers from Yu and Zhang (2005). Polymerase Chain Reactions (PCR) were carried out as in Patou et al. (2009a), with hybridisation temperatures at 50 °C for Cytb and ND2, 61 °C for CR, and 59° for FGB. PCR products were then purified and sequenced in both directions on an automated DNA sequencer by Genoscope (Evry, France) and Eurofins (Ebersberg, France). Sequences obtained from DNA extracted from museum samples were amplified and sequenced twice to ensure their quality and authenticity. Sequences were edited, assembled and aligned manually, using Bioedit (version 7; Hall, 1999).

Each gene was analysed individually and combined. Phylogenetic analyses were performed via Bayesian Inference (BI), using MrBayes 3.2 (Ronquist et al., 2012) and Maximum Likelihood (ML), using MEGA6 (Tamura et al., 2013). For ML, the best-fitting model was estimated prior to the analyses using MEGA6, following the Akaike information criterion (AIC). The selected model was then implemented in the ML analyses, in which node robustness was assessed through 1000 bootstrap replicates. For BI, we used Reversible Jump Markov Chain, to sample across the 201 substitution models, and gamma distribution (Lsetnst = mixed rates = gamma option) to sample the posterior distribution of trees and to take into account the substitution model uncertainty. We used default priors for branch lengths and ran the chains for 10,000,000 Metropolis-coupled MCMC generations, with trees sampled every 1000 generations, and a burn-in of 25%.

Trees were visualized and edited using FigTree 1.4.0 (Rambaut, 2012). We compared resulting topologies and their node support; nodes were considered as supported when posterior probabilities were $\geq$0.99 and bootstrap values were $\geq$70%. Genetic distances and Neighbor Joining trees (NJ, Saitou and Nei, 1987) were computed using MEGA6.

We used DNAsp 5.10 (Librado and Rosas, 2009) for defining haplotypes and to compute genetic diversity (haplotype and nucleotide diversity), and Network 4.6 (http://www.fluxus-engineering.com) to construct haplotype median-joining networks (Bandelt et al., 1999).

Results

We obtained new sequences of the four markers for 31 individuals of *U. auropunctata*, *U. edwardsii* and *U. javanica* (GenBank numbers KY346541 to KY346606); other sequences were from previous studies (see Table 1).

The fragment length, number of variable sites, number of parsimony informative sites, and number of individuals used in the phylogenetic analyses (including outgroups), were: FGB (579/22/7, n = 29; Model T92+G); Cytb (1140/251/137, n = 45; Model GTR+G+I); ND2 (276/57/35, n = 36; Model TN93+I); CR (431/90/68; n = 19, model HKY+G+I); Cytb+ND2 (1416/308/172, n = 45; model GTR+G+I); Cytb+ND2+FGB (GTR+G+I; 1995/330/179, n = 45). All phylogenetic trees (with all methods), although poorly resolved, retrieved three main clades corresponding to the three species *U. javanica*, *U. auropunctata* and *U. edwardsii* (Fig. 2). The resolution within these clades was low. However, the individuals from southern China and Hong Kong clustered with *U. javanica*. Within *U. javanica* (Figs. 2 and 3), the Javan individuals were a sister group to all the remaining individuals; those from northern Thailand formed a well-supported group, which was sister to a clade that comprised individuals from China, Hong Kong and Vietnam and a group from central and southern Thailand. Within *U. auropunctata* (Fig. 2), two clades were retrieved: one comprising Myanmar individuals, and one containing all others (Bangladesh, Pakistan, and introduced populations that were said to come from India).

The Cytb haplotype network for *U. javanica* (Fig. 4; 737 sites; n: 12; h: 9; Hd: 0.9091; Pi: 0.01589; S: 47; Eta: 49) showed a haplogroup of three haplotypes (H2, H3, H9) from Vietnam, southern China and Hong Kong, each separated by one mutation. Another haplotype from Vietnam (H8) was separated by five mutations from this group. Thailand specimens were represented by four haplotypes; three from southern and central Thailand (H4, H6, H7) separated from the haplotype from northern Thailand (H5) by 13–21 mutations. The haplotype from Java (H1) was separated by 18 mutations from the closest haplotype (H9).

The Cytb haplotype network for *U. auropunctata* (Fig. 5; 704 sites; n: 16; h: 14; Hd: 0.942; Pi: 0.00935; S: 31; Eta: 31) showed two main groups: one containing two haplotypes from Myanmar (H7, H8), and another comprising the haplotypes from Pakistan, India, Bangladesh, and introduced areas (Croatia, Fiji, Guyana, Japan, and the Virgin Islands) that was separated by 28 mutations from the first. The haplotypes from Fiji (H4) and the Virgin Islands (H3) were close to the ones from Bangladesh (H9/H10), and the haplotype from Croatia (H11) was close to those from Pakistan (H12, H13). The haplotypes from Japan (H1, H2) were distant from all the