Short Note

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First record of *Mus cookii* (Cook's mouse) from Pothwar, Pakistan: a probable case of range extension?

<https://doi.org/10.1515/mammalia-2017-0130>

Received October 5, 2017; accepted April 6, 2018; previously published online May 23, 2018

Abstract: The Southeast Asian species *Mus (Mus) cookii* Ryley 1914 has been previously reported from southern China, Nepal, northeastern and peninsular India, Myanmar, north and central Thailand and Vietnam. A cytochrome *b* (*Cytb*) gene analysis revealed the presence of *M. cookii* which significantly expands the known western range of this species. However, it is unclear whether the *M. cookii* population in the Pothwar region represents a historical or recent westward range expansion or, on the contrary, a relictual source population that further experienced an historic eastward range expansion into South-east Asia.

Keywords: *Mus*; Northern Punjab; Pakistan; Pothwar; range extension.

The subgenus *Mus* is traditionally divided into three main clades: the house mouse clade, the Palearctic or Eurasian clade and the Asian clade (Lundrigan et al. 2002). The Asian clade includes three species: *Mus caroli* Bonhote 1902, *Mus cervicolor* Hodgson 1845 and *Mus cookii* Ryley 1914. Lundrigan et al. (2002) described this clade as monophyletic based on the study of paternally, maternally and biparentally inherited genes. Monophyly of the Asian clade was confirmed by analysis of morphological data, DNA/DNA hybridization and phylogenetic studies of mitochondrial,

Y-linked and autosomal markers (Lundrigan et al. 2002, Chevret et al. 2003, Shimada et al. 2010). Nonetheless, the Asian clade is not as well studied as the Palearctic clade (Lundrigan et al. 2002) as geographic ranges of Asian species are incompletely known, and geographic variation within the species has not been assessed. On the basis of mitochondrial and nuclear gene analyses, Suzuki et al. (2004) identified three distinct species group in the subgenus *Mus*: a *Mus booduga* Gray 1837 species group, including *Mus terricolor* Blyth 1851 and *Mus fragilicauda* Auffray et al. 2003; a *Mus musculus* species group, also including *Mus macedonicus* Petrov and Ruzic 1983, *Mus spicilegus* Petenyi 1882 and *Mus spretus* Lataste 1883 and a *M. cervicolor* species group, also including *M. caroli* and *M. cookii*. Mitochondrial and nuclear gene analyses by Chevret et al. (2005) resulted in conflicting topologies about the relationships between the three Asian species of the *M. caroli*, *M. cervicolor* and *M. cookii* as the nuclear dataset supports a *cervicolor-cookii* association, while in contrast, the 12S ribosomal RNA (rRNA) gene indicates a close association between *caroli* and *cookii*; *M. cookii*, commonly known as Cook's mouse, is naturally distributed in two distinct South-east Asian populations, one found mainly in Bangladesh, Bhutan, northeastern India and Nepal (Molur et al. 2005) and the second in central and eastern Myanmar, southern China, Thailand and Vietnam (Smith and Xie 2008) (Figure 1). Moreover, Agrawal (2000) described the Indian populations of *M. cookii* as disjunct with one part found in south peninsular India (*M. cookii cookii*) and the other part distributed in northeastern India (*M. cookii nagrum* Thomas 1921). Habitats commonly used by *M. cookii* include mountains, hills, grasslands, rice fields and pine and deciduous forests (Marshall 1977, Aplin et al. 2016).

Mice specimens (180) were collected in 6912 trapping nights with a trapping success of 2.60% (for mice species) from the Pothwar region (32°33'–34°26' N; 71°89'–73°37' E) located in Northern Punjab, Pakistan during December, 2011–December, 2014. Pothwar is a region bounded by the Murree hills and foothills of the Himalayas in the north,

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Figure 1: Geographical range of *Mus cookii* according to the International Union for Conservation of Nature (IUCN) red list version 2016-3. <http://maps.iucnredlist.org/map.html?id=13958> and the new locality record from Pothwar (Koont Farm), Pakistan (33° 06' 58.0"N, 73° 00' 52.2"E).

the Jhelum River in the east, the Salt range in the south and the Indus River in the west. The area of the plateau is about 13,000 km² and the elevation ranges from 305 to 610 m above sea level (Nadeem et al. 2012). It is a humid subtropical region and the rainfall varies from 300 mm southward to 500 mm northward (nearer to the Himalayan foothills) and most of the rains are (60–70%) received in the monsoon season (Shafiq et al. 2005). The mean summer temperature is about 40°C and winter is 25°C. About 110,600 ha area of the plateau is cultivated (GOP, 2002–2003) and 96% of cultivation depends on rain. The major crops are wheat, groundnut, sorghum, barley, legumes and various types of vegetables and fruit.

Standard body and skull measurements of the collected samples were taken and compared to those provided by Marshall (1977). Genomic DNA from the ethanol preserved tail tissue was isolated using the protocol developed following the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA), followed by polymerase chain reaction (PCR) using a pair of primers (5'CATGAAAAATCATCGTTGTAA 3' and 5'TCTTCATTTTGGTTTACAAGACCA 3'; Torrance 1997). The entire cytochrome b (*Cytb*) (1140 bp) was sequenced using the same primer sets as used for PCR. Cleaned PCR products were sent to Australian Genome Research Facility Ltd. (AGRF) for sequencing in both

forward and reverse directions. Sequences were verified by visual inspection of chromatograms. Sequence alignments were performed using MAFFT 5 implemented in Geneious 6.1.5. (<http://www.geneious.com>, Kearse et al. 2012).

Out of the 180 collected *Mus musculus* samples, one *Mus cookii* sample was recovered. The Basic Local Alignment Search Tool (BLAST) was used to initially identify this *M. cookii* sequence which resulted in 100% match with the other *M. cookii* sequences available in GenBank. A phylogenetic tree for the *Cytb* gene sequences was constructed by the neighbor-joining (NJ) method using Geneious 6.1.5. For NJ tree construction, the *M. cookii* sequence from the present study was compared with other *M. cookii* sequences available in GenBank (accession numbers are given in Figure 2). The NJ tree shows that the new *M. cookii* sequence from the present study is closely related to the other *M. cookii* lineages represented by previous sequences present in GenBank (Figure 2). The DNA sequence from this specimen has been submitted to the GenBank database (accession no. KX790791). The pelage as well as the body and cranial characters (Table 1) were also similar in all significant features to *M. cookii* reported by Marshall (1977). This specimen had dark gray dorsal fur and light gray ventral fur while the same pattern was noted true for the tail color. Marshall (1977) described *M. cookii* as larger

Figure 2: Neighbor-joining tree showing the position of *Mus cookii* from Pakistan (node shown in red) among related species and other *M. cookii* sequences from its previous range.

mice having stiff fur of dark gray color or short fur with dark or pale coloration. Our specimen (SB 15, Accession no. KX790791) satisfies all these diagnostic characters.

This specimen of *Mus cookii* was captured from the wheat crop field of the University Research Farms Koont of PMAS-Arid Agriculture University Rawalpindi (33° 06' 58.0"N, 73° 00' 52.2"E) on 30th December 2011 (Figure 1). The total area of Koont Farm is about 250 acres (101.17 ha) and it is located in Rawalpindi District on Chakwal road near Dhudial village, about 80 km from Rawalpindi city. The soil is predominantly sandy loam and sandy clay loam with variations of loamy sand; there is a dominance of sand particles in the soil (Shafiq et al. 2005) along with alkalinity and sodicity. Therefore, these soils are deficient in organic matter and phosphorus, which results in sparse

Table 1: Comparison of body weight (g), body and cranial measurements (mm) of *Mus cookii*, captured from Pothwar, Pakistan and *Mus cookii cookii* and *Mus cookii nagarum* from Marshall (1977).

Variable	<i>M. cookii</i> (present study)	<i>M. c. cookii</i> (Marshall 1977)	<i>M. c. nagarum</i> (Marshall 1977)
BW	22.0	23.2	15.0
HBL	96.0	92.7	73.4
TV	85.2	83.5	73.8
HF	18.6	19.2	17.2
EL	15.0	—	—
GSL	22.5	24.4	21.9
BBC	10.3	10.5	9.5
DBC	7.0	—	—
ZB	2.0	2.2	2.1
NL	8.9	9.0	8.3
IFL	4.9	5.2	4.8
IOB	3.8	3.9	3.5
ML	10.7	—	—
UMRL	3.9	—	—
LMRL	3.8	—	—
M1L	2.0	—	—

BW, Body weight; HBL, head and body length; TV, tail length; EL, ear length; HFL, hind foot length; GSL, greatest skull length; BBC, breadth of the braincase; DBC, depth of the braincase; ZB, zygomatic breadth; NL, nasal length; IFL, incisive foramen length; IOB, inter orbital breadth; ML, mandible length; UMRL, upper molar tooth row length; LMRL, lower molar tooth row length; M1L, first molar length. Agrawal (2000) also provides measurements for the Indian subspecies. Thus, two subspecies of *M. cookii* occur in India.

Key to subspecies of *Mus cookii*

Size large, occipitonasal length of skull more than 23 mm.
M. c. cookii

Size small, occipitonasal length of skull less than 23 mm.
M. c. nagarum

(i) *Mus cookii cookii* Ryley 1914. *Mus cookii* Ryley, 1. Bombay nat. Hist. Soc., 22: 664 (Shan State, Myanmar).

Measurements " (vide Ellerman 1961).

External, 22 ex.: HB 70–99 (84 ± 9); TI 60–96 (81 ± 9); Hf 15–21 (18.5 ± 1); E 12–17 (15 ± 1).

Cranial: 10 ex.: on/23.4. 25.8 (24.2 ± 0.8); p 11.2–13.4 (12.3 ± 0.5); d 5.6–7.4 (6.25 ± 0.45);

nl 3.7–4.1 (3.95 ± 0.15); apj 4.8–6.0 (5.2 ± 0.3).

Distribution (Map) (2): India (Naga Hills). Also reported from Myanmar (Kabaw valley), China (Yunnan), Thailand, Laos and Vietnam.

(ii) *Mus cookii nagarum* (Thomas)

1921. *Leggada nagarum* Thomas, J. Bombay nat. Hist. Soc., 27: 597 (Golaghat, Assam, India).

1923. *Leggada palnica* Thomas, J. Bombay nat. Hist. Soc., 29: 87 (Shambagama c 1830 m, Palni Hills, Tamil Nadu).

Measurement:

External:

nagarum: 32 ex: HB 64–86 (74.4 ± 5.5); TI 56–82 (68.4 ± 7); Hf 15–19 (16 ± 1.6); E 10–16 (13 ± 1.0.5).

palnica: 10 ex: HB 65–95 (76.4 ± 9); TI 71–90 (78.2 ± 6); Hf 16–18 (17 ± 0.5); E 13–14 (13.5 ± 0.5).

Cranial:

nagarum: 19 ex: onl 19.2–22.4 (21.4 ± 1.3); p 9.0–11.8 (10.6 ± 0.7); d 4.6–6.1 (5.34 ± 0.45);

Table 1 (continued)

m 2.9–3.9 (3.5 ± 0.3); apf 4.1–5.4 (4.7 ± 0.3).
palnica: 7 ex: onl 21.0–22.9 (21.9 ± 0.8); p 10.5–11.5 (11.05 ± 0.4)
 d 5.4–5.9 (5.55 ± 0.2);
 m 3.5–3.6 (3.5 ± 0.05); apf 4.5–5.3 (4.8 ± 0.3).
 Distribution (Map 12): India: Arunachal Pradesh (Mishmi Hills),
 Assam (Golaghat, N. Kamrup), Meghalaya (Khasi and Jaintia Hills),
 Manipur (Chandel dist.),
 Nagaland, West Bengal (Darjiling), Maharashtra (Pune), Karnataka
 (Coorg, Mysore), Kerala (Cannonore) and Tamil Nadu (Palni Hills,
 Kodaikanal) (Ellerman 1961, Marshall 1977).

vegetation in the area. In Koont Farm, scattered trees of beri (*Ziziphus jujuba*), tahli (*Dalbergia sissoo*), dhareek (*Melia azadirachta*) and desi kinar (*Acacia nilotica*) were observed; other wild plants were oak (*Calotropis procera*), poli (*Carthamus oxycantha*), dub (*Imperata cylindrica*), bhang (*Cannabis sativa*) and common grass (*Cynodon dactylon*). The important crops of the Koont Farm are pearl millet (*Pennisetum glaucum*), til (*Sesamum indicum*), wheat (*Triticum aestivum*), groundnut (*Arachis hypogaea*) and sorghum (*Sorghum bicolor*). Some patches of experimental maize (*Zea mays*) were also present. Other rat and mice species captured from the various crops/sites of the farm were *Nesokia indica*, *Tatera indica*, *Rattus rattus*, *Golunda ellioti*, *Bandicota bengalensis* and *Mus musculus*.

This study demonstrates for the first time the presence of *Mus cookii* in Northern Pakistan, thus revealing a major westward expansion of the range of this species of about 1360 km (from central Nepal to the Pothwar region, Pakistan). *Mus cookii* inhabits a wide variety of habitats. In South Asia, it is found in subtropical dry deciduous forests, temperate coniferous forests, grasslands and arable land near *Lantana* bushes (Molur et al. 2005). Conversely, in Southeast Asia, *M. cookii* is found in forest areas and occasionally in moderately disturbed areas such as upland gardens in forests. The prevalence of similar subtropical habitats and deciduous forests and probably the mountainous range of the Himalayan foothills in the Pothwar region of Northern Punjab may have favored the distribution of the species to this region.

Marshall (1977) and Agrawal (2000) identified two subspecies of *Mus cookii*: *Mus cookii cookii* Ryley 1914, a larger sized mouse with molar tooth row of 4 mm and *Mus cookii nagrum* Thomas 1921, a moderate sized mouse with molar tooth row of about 3.5 mm. The two subspecies are sympatric in northeastern India and Nepal, while the intermediate forms are found in Myanmar. The new specimen collected during this study was identified as *M. c. cookii* based on comparison with Marshall (1977) (Table 1). Agrawal (2000) also identified *M. c. cookii* with

a skull length greater than 23 mm and *M. c. nagrum* with a skull length less than 23 mm. Musser and Carleton (2005) described *M. cookii* as a possible species complex, but considered the name *Mus nagrum* a synonym of *M. cookii* and recommended further research and review of museum specimens for clarification. There are many morphological differences between the disjunct populations of *M. c. nagrum* in northeastern India and *M. cookii* in Southeast Asia (Aplin et al. 2016).

As far as the occurrence of *Mus cookii* in Pakistan is concerned, Marshall (1977) discussed the morphological similarity of *Mus cervicolor* and *M. cookii*, and also reported their coexistence in Myanmar. Similarly, *M. cervicolor* is also known to occur in Pakistan (Taber et al. 1967). The present study may suggest that the absence of *M. cookii* from historic faunal surveys of Pakistan may represent a case of misidentification of *M. cookii*. Or it can be the case of a very recent major range extension of *M. cookii*. This area was linked in the past through the ancient silk/trade route for centuries to Afghanistan, Iran, China and Central Asia. Moreover, Muslim invaders from Iran and Afghanistan also conquered this region frequently during the 12th–16th century. Therefore, it is more likely that *M. cookii* may be present from that time but not explored by the researchers. So in order to reveal the true identity of different *Mus* taxa inhabiting this region, a more extensive sampling throughout Pakistan is recommended involving robust specimen identification and genetic analyses of the available type specimens to match the name with the genotype.

Acknowledgments: This study was partly funded by the Pakistan Science Foundation (project no. PSF/Res/P-PMAS-AAUR/Bio.446) and Higher Education Commission of Pakistan (under Ph.D. Fellowship Program). We are most grateful to Andrew Wiewel, University of Adelaide, South Australia for his valuable comments and suggestions during the preparation of this manuscript.

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