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First investigation on the diet of the eastern grass owl during the nesting period in Thailand

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Abstract. The eastern grass owl *Tyto longimembris* was first detected in Thailand in July 2006 at Nong Lom, a grassland in open peat swamp located in the south part of Nong Bong Khai Non-hunting Area, Chiang Rai. Here, it is considered to be a rare resident. At this site, we studied the diet of eastern grass owl by analysing regurgitated pellets collected at their nests during the breeding season from December 2010 to February 2011. We collected 67 pellets from five nests and identified 33 mammal skulls. To identify prey species, DNA was extracted from skulls and was analysed based on molecular techniques. The dietary remains consisted of three murids (Muridae), with the house rat *Rattus rattus* the dominant species detected (16 skulls, 48.5 % occurrence), and the remainder being Asian house mouse *Mus musculus* (13 skulls, 39.4%) and ricefield mouse *Mus caroli* (4 skulls, 12.1%).

Key words. eastern grass owl, diet, pellet, nocturnal raptor

INTRODUCTION

Species within the family Tytonidae (barn owls) are nocturnal or sometimes crepuscular predators that mainly hunt small nocturnal mammals, especially rodents, but sometimes also birds, reptiles and amphibians (Colvin & McLean, 1986; Britton & Rose, 1999; Debus et al., 2004; Shehab, 2005; Clulow et al., 2011). Typically they nest in tree hollows. However, two species of grass owl, the African grass owl *Tyto capensis* and the eastern grass owl *T. longimembris*, roost and nest in tall grasslands or on the ground in ground-covering plants (Maciejewski, 1996; Hamid et al., 2008). The African grass owl has an extremely large range in Africa, while the Eastern grass owl is distributed throughout southern China, Nepal, India, Myanmar, Thailand, Vietnam, the Philippines, Indonesia, New Guinea, Australia and New Caledonia (BirdLife International, 2012, 2014). Formerly, the eastern grass owl had been considered conspecific with African grass owl but the two were subsequently classified as distinct species (Baker, 1993; del Hoyo et al., 1999; Sinclair et al., 2002; König & Weick, 2008). In Thailand, the eastern grass owl was detected for the first time on 29 July 2006 at Nong Lom, a small area of marsh grasses near the Nong Bong Khai Non-hunting Area, Chiang Rai province where it is now considered a rare resident (Round, 2007; Wongchai, 2007; Robson, 2009).

In Thailand, few studies have reported on the biology of birds of prey, and the eastern grass owl is one of the least known species. Current knowledge of the eastern grass owls in Thailand is limited to their breeding habitat usage from the swamp grassland of the Chiang San basin (Kasorndorkbua et al., 2008).

This study focuses on the food habits of the eastern grass owl during the breeding season in Thailand. This basic knowledge is important for the management and conservation of this population and their habitat.

MATERIAL AND METHODS

Study area. Eastern grass owl nests were found in a swamp grassland of a size of approximately 2 km^2, locally called "Nong Lom", at 20°11′N, 99°58′E, which is located in Mae Jan district, Chiang Rai province, Northern Thailand. This is the south part of the Chiang Saen Basin Important Bird Area (Bird Conservation Society of Thailand, 2004). The grassland is part of a large wetland area (approximately 110 km^2) used year-round by the local community for traditional fishing, animal hunting and livestock grazing. The predominant grass species in the area used for nesting are southern cutgrass *Leersia hexandra* and cogon grass *Imperata cylindrica* (Kasorndorkbua et al., 2008). This area is also used as the winter roosting site of four species of harriers; eastern marsh harrier *Circus spilonotus*, pied harrier *C. melanoleucos*, hen harrier *C. cyaneus* and western marsh

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harrier *C. aeruginosus*, with approximately 200 individuals roosting at the site (Round, 2005; Thai Raptor Group, 2006).

Pellet collection. Five eastern grass owl nests with a total of 25 nestlings were found during the breeding season of early December 2010 through late February 2011. After all the nestlings had fledged, we collected pellets and other remaining prey items from the nests. Some pellets had begun to decompose and were damaged, thus only 67 regurgitated pellets were collected. All pellets were dried and measured in width, length and depth using a vernier caliper (with an accuracy up to 0.05 mm) and weighed using electronic scales (with an accuracy up to 0.01 g). Then, all pellets were carefully dissected with tweezers and all bone material from each pellet was separated out for identification (based on methods used by Dickman et al., 1991).

Circus harrier pellets were also found in the same habitat as the eastern grass owl, but there are some important differences between their pellets. The size of harrier pellets overlaps with that of the owls, but in most cases the pellets are much smaller, rounder in shape, with a similar width to length with smooth texture of mammal hair. Owl pellets were usually 1.5 times longer and elliptically shape (Fig. 1). The fact that we collected owl pellets from directly within the nests also precludes any possibility of accidental harrier pellet collection.

Prey identification and laboratory analysis. We used a molecular technique to investigate owl diet using genetic material extracted from skulls of their prey. This technique was used because there is limited reference material on mammal morphology or museum specimens in Thailand to allow for visual identification of the owls' prey.

All skull samples from pellets were ground and extracted by using modified proteinase K and a standard phenol-chloroform protocol for total genomic DNA (Sambrook et al., 1989; Shao et al., 2009). The polymerase chain reaction (PCR) was used to amplify a region of the 12S mitochondrial gene with the primers 12S1L:CAAACTGGGATTAGATA CCCCACTAT and 12S2H: AGGGTGACGGGCGGTGTG (Kocher et al., 1989).

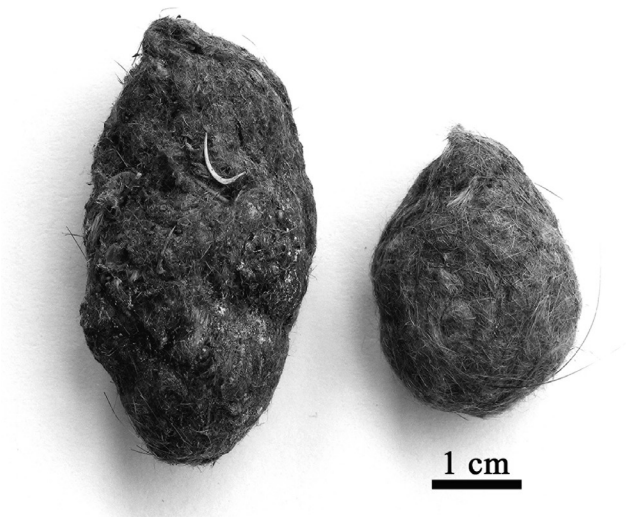

Fig. 1. Typical pellet of an eastern grass owl (left) and a *Circus* harrier (right).

The amplification was performed in a 25 µl volume reaction containing 1 µl (100 ng) of genomic DNA, 17.5 µl of dH_2O, 2.5 µl of 10× PCR buffer, 1 µl (3 pmol) of each primer, 1 µl (0.1 mg ml^{-1}) of BSA, 1 µl (10 mM) of dNTP and 0.2 µl (5U μl^{-1}) of Taq polymerase with the following procedures: initial denaturation step with 5 min at 95°C, 35 cycles of denaturation 30 s at 95°C, annealing for 30 s at either 55°C, extension for 30 s at 72°C. Final extension at 72°C was conducted for 5 min. All PCR products were purified using the Qiagen PCR Purification Kit (Qiagen, Germany) and finally sequenced (Macrogen, Korea). Nucleotide sequences were aligned using ClustalX 1.81 (Thompson et al., 1994; Thompson et al., 1997) with default parameters, and then optimised by eye in MEGA 5.0 software (Tamura et al., 2011). Phylogenetic analyses among haplotypes were conducted using maximum likelihood (ML) with 2000 bootstrap replicates using unweighted characters (Felsenstein, 1985) and were performed in MEGA 5.0 software.

RESULTS

Pellet characteristics. A total of 67 eastern grass owl pellets were collected and they ranged in length from 2.18–5.53 cm (Fig. 2), having the average size of 4.18 cm (±0.7 SD) × 2.57 cm (±0.38 SD) × 2.00 cm (±0.33 SD) with an average dry weight of 4.71 g (±1.34 SD). A total of 33 pellets contained only one skull each (type A) while 34 pellets contained only postcranial skeletal material (type B) (Table 1).

Prey species. A 382 bp stretch of the 12S mitochondrial gene was analysed from all 33 skull samples plus 5 reference sequences from Genbank (accession numbers see Fig. 3). The resulting phylogenetic tree (Fig. 3) shows that there are three different species in the owl diet, all of which belong to the family Muridae: house rat *Rattus rattus*, Asian house mouse *Mus musculus* and ricefield mouse *Mus caroli*. Overall nucleotide diversity (π) was 0.151. The pairwise genetic distances of 12s rRNA gene sequences calculated with the Kimura 2- parameter model were 0.000 to 0.394. Average Kimura 2-parameter mean genetic distances within *R. rattus*, *M. caroli* and *M. musculus* were 0.073 (±0.009 SD), 0.016 (±0.005 SD) and 0 respectively, and between species are shown in Table 2. The nucleotide frequencies were A = 33.99%, C = 19.36%, G = 21.18% and T = 25.47%. The transition/transversion rate ratios were k_1 = 1.723 (purines) and k_2 = 1.507 (pyrimidines). The overall transition/transversion bias was R = 0.782. Average genetic diversity, mean genetic diversity between species and coefficient of differentiation were 0.180, 2.148 and 1.194 respectively.

Of three rat species, the house rat was the dominant food of the owls with16 of 33 skulls (48.5%), followed by Asian house mouse with 13 skulls (39.4%) and ricefield mouse with four skulls (12.1%) (Table 3; Fig. 3).

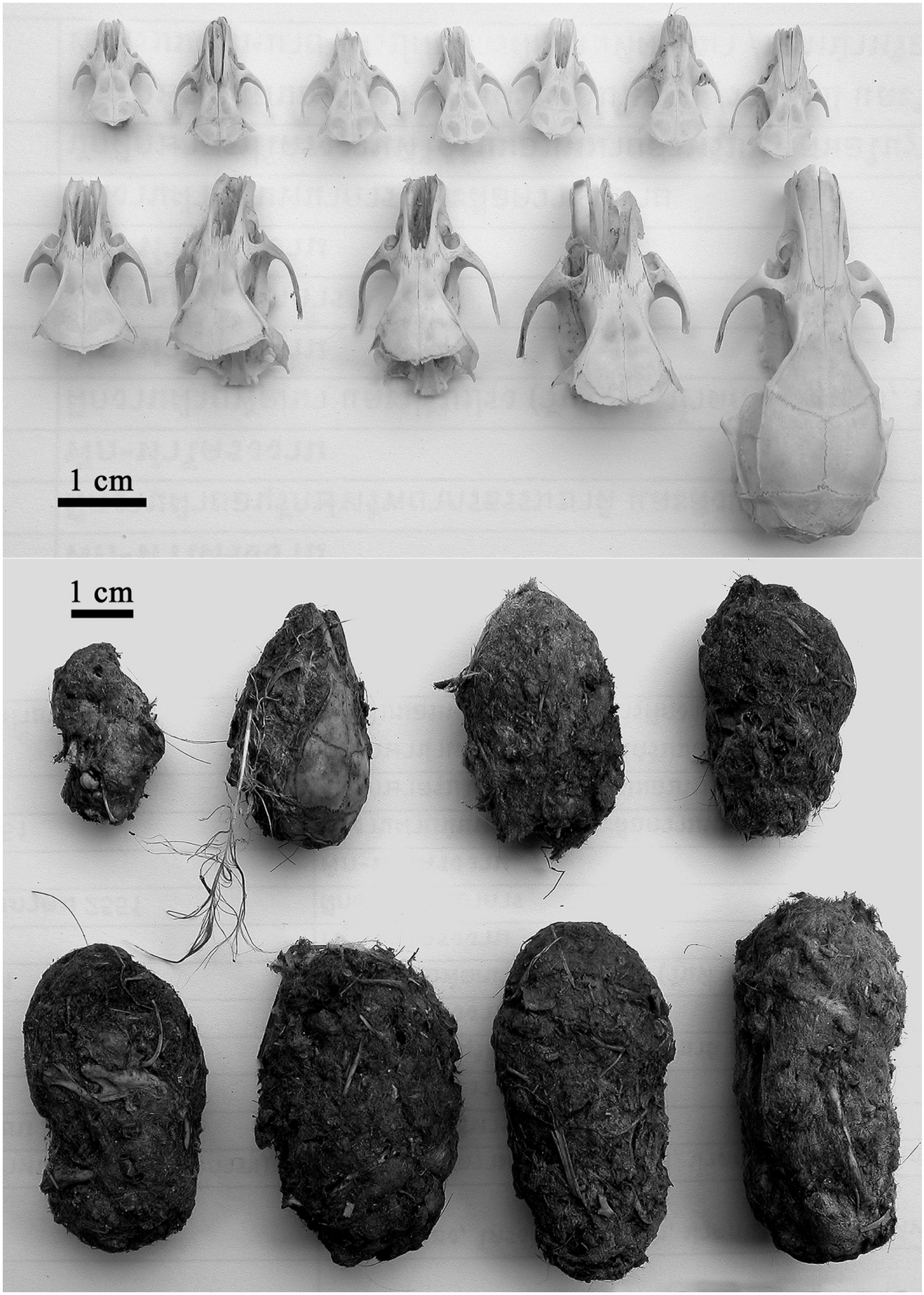

Fig. 2. Different sizes of pellets and prey's skulls of eastern grass owl.

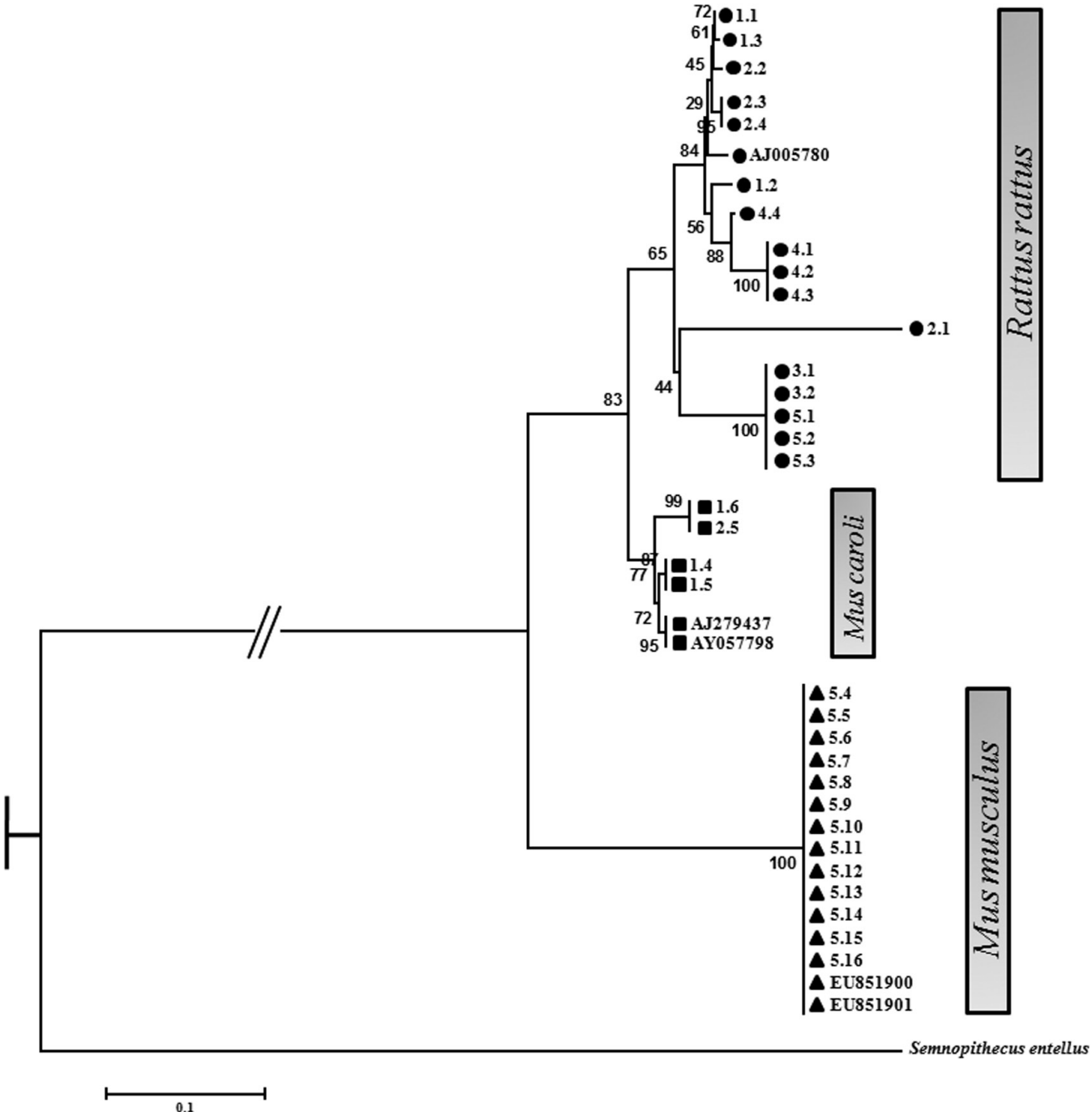

Fig. 3. Cladogram showing phylogenetic relationships of the sequenced mitochondrial DNA of three rodent prey species of the eastern grass owl.

DISCUSSION

From this study, three species of murids (Muridae) comprising one rat and two mouse species were detected in the diet of the eastern grass owl during the breeding period. Apart from rodents, there were no other prey species detected. However, we did find a small number of wings and tails of barn swallows *Hirundo rustica* at the owl's nest, so it is likely that the barn swallows were eaten by the owls because they commonly roost in the same area.

Although the eastern grass owl is distributed across Asia, knowledge of its biology and ecology is limited. One dietary study in southern Taiwan (Lin et al., 2007) revealed that mammal species (95.1%) were the predominant dietary items, while the remaining (4.9%) were birds. Their study found that rats were the main food source, especially the lesser rice-field rat *Rattus losea* followed by ricefield mouse *Mus caroli*, the latter species having also been consumed by the eastern grass owl in Thailand. In addition to consuming rats and mice, Lin et al. (2007) reported other mammals as dietary items, such as Formosan blind mole *Mogera insularis*, house shrew *Suncus murimus*, shrew *Crocidura* spp. and juvenile Formosan hare *Lepus sinensis* but similar species were not found as dietary items of eastern grass owls in Thailand. The high dietary diversity of the eastern grass owl in Taiwan (nine species) was likely due to the great variety of habitat types available at that study site. The habitat types around nest

Table 1. Dimensional characteristics of eastern grass owl pellets (means±SD, with range in parentheses). Type A = Pellet with only one mammal skull and Type B = Pellet containing only postcranial material.

Pellet type	Length (cm)	Width (cm)	Depth (cm)	Dry weight (g)
Type A (*n*=33)	4.44±0.61 (2.99-5.53)	2.70±0.35 (2.99-3.36)	2.11±0.28 (1.28-2.71)	4.99±1.46 (2.10-8.54)
Type B (*n*=34)	3.93±0.7 (2.18-4.79)	2.45±0.36 (1.62-3.28)	1.89±0.34 (1.21-2.36)	4.44±1.17 (2.07-6.89)
Total (=67)	4.18±0.7 (2.18-5.53)	2.57±0.38 (1.62-3.36)	2.00±0.33 (1.21-2.71)	4.71±1.34 (2.07-8.54)

Table 2. Mean genetic distances between rodents detected from eastern grass owl pellets in this study by Kimura 2-parameter model (K2P).

Species	*Rattus rattus*	*Mus caroli*	*Mus musculus*
Rattus rattus	0.000		
Mus caroli	0.111	0.000	
Mus musculus	0.326	0.259	0.000

Table 3. Dietary remains of the Eastern grass owl showing number and size differences between species.

Common name	Scientific name	Number of pellet(s)	Percent occurrence	HB* (mm)
House Rat	*Rattus rattus*	16	48.5	105–215
Asian House Mouse	*Mus musculus*	13	39.4	65–90
Ricefield Mouse	*Mus caroli*	4	12.1	65–85
	Total	33	100.0	

*= Head and body length is measured from the front of the nose to the anus when the animal is stretched out (Lekagul & McNeely, 1977; Francis, 2008)

sites included large shrubland, rice paddies, sugarcane fields, mango orchards, bamboo forest and settled landscape. It is considerably different from the habitat of the eastern grass owl from Nong Lom in Thailand where most habitat was tall grassland surrounded by wetlands and orange orchards, located approximately 1 km northwest from the nest sites.

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