

## Short Communication

## Assessment of rodent communities in two provinces of Cambodia

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*Paper submitted 28 September 2017, revised manuscript accepted 30 November 2017.*

Rodents play an important role in regulating ecosystems, in particular through seed dispersion and predation, and constitute a prey for mesopredators. However, rodents are considered pests in rural areas because they can cause significant damage to crops, especially during episodes of sudden population growth (Singleton, 2003). Rodents are also carriers or reservoirs for numerous zoonotic diseases that represent a serious threat to human health (Meerburg *et al.*, 2009; Luis *et al.*, 2013; Olival *et al.*, 2017). Multi-site assessments of rodent communities are consequently needed to understand the implications of various spatio-temporal factors on these ecosystem services, the epidemiology of rodent-borne diseases, and other interactions with humans.

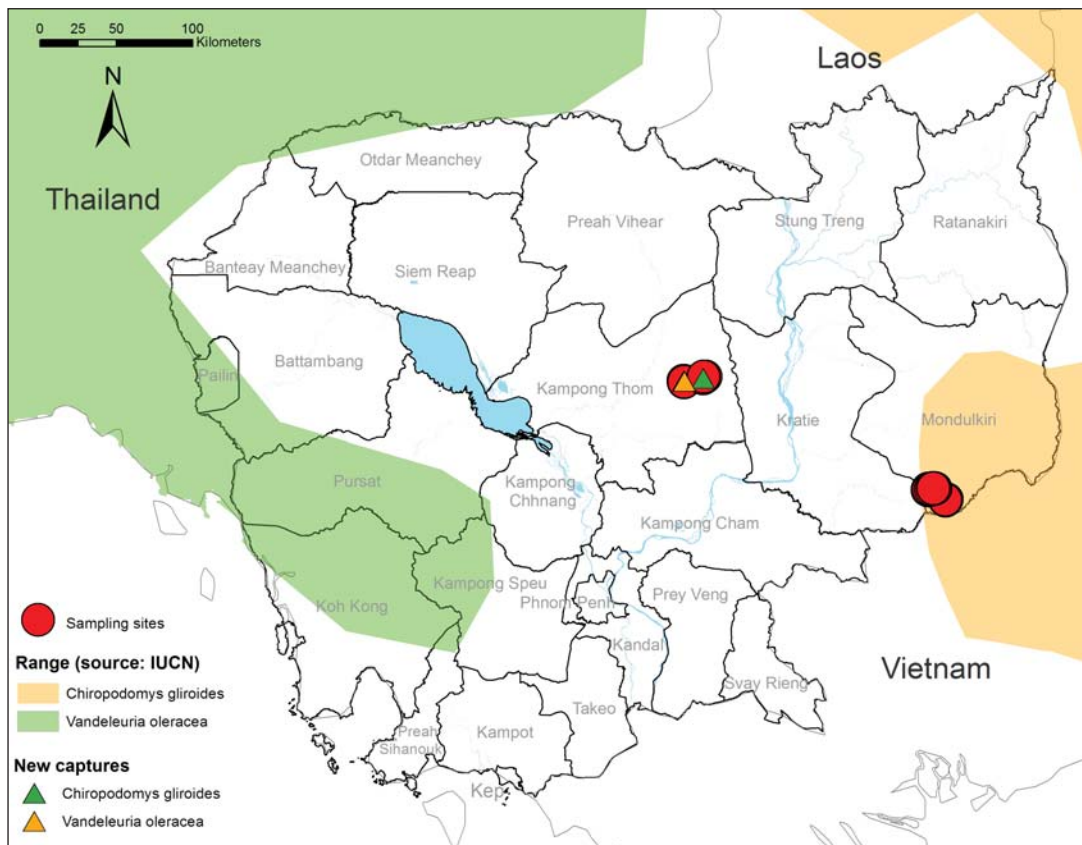
Between June 2015 and April 2016, field surveys were conducted to assess rodent community composition at five sites in Cambodia: the Orona, Pouy Doem Svay and Ochra villages in Keo Siema District, Mondulhiri Province, and the Sro Lov Sroung and Rom Chek villages in San Dan District, Kampong Thom Province (Fig. 1). Each site was visited twice, once during the wet season (June–September 2015) and once in the dry season (January–

April 2016). During each visit, 150 traps were deployed in a variety of habitats (including evergreen or semi-evergreen forest, and cultivated lands) whose locations were recorded using handheld GPS units (Garmin 60Cx, Garmin 60Csx, USA). Rodents were trapped using locally made and non-lethal Havahart traps for eight consecutive nights in each season at each site (=12,000 trap nights in total). All of the study procedures were approved by the Wildlife Conservation Society's Institutional Animal Care and Use Committee, under protocol #15:04.

Captured individuals were marked with a one millimetre ear notch. These skin samples were stored in 95% alcohol and placed in liquid nitrogen. Each animal was given a unique identification code and photographed before release. Morphological criteria (Wilson & Reeder, 2005; Francis, 2008; Chaval, 2011) and molecular analyses were employed for species identification. DNA was extracted from the skin samples using the Qiagen DNeasy® Blood & Tissue Kit according to the manufacturer's instructions. The primer set of BatL5310 (5'-CCTACTCRGCCATTTTACCTATG-3') and R6036R (5'-ACTTCTGGGTGTCCAAAGAATCA-3') were used

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CITATION: Fillieux, C., Chea S., Ramassamy J.-L., Hul V., Olson, S., Fine, A., Dussart, P., Phimpraphi, W., Horwood, P.F. & Pruvot, M. (2017) Assessment of rodent communities in two provinces of Cambodia. *Cambodian Journal of Natural History*, 2017, 147–152.



**Fig. 1** Study locations in Kampong Thom Province and Mondul Kiri Province, 2015–2016.

to amplify a 750 base pair fragment of the Cytochrome c oxidase subunit I (COI) gene, as described by Blasdel *et al.* (2015). Amplicons were sequenced using the Sanger method at a commercial facility (Macrogen, Seoul, South Korea). Species identifications were obtained by comparing DNA sequences to existing records. This process was facilitated by previous DNA barcoding efforts for rodent species in the region (e.g., CERoPath: [www.ceropath.org](http://www.ceropath.org)).

Where results of molecular identification were not consistent with morphological characteristics (five individuals) or when DNA sequences were not available due to missing skin samples (25 individuals), species determinations relied on morphological identification validated using archived photographs. Due to missing data, 13 individuals could only be identified to genus (11 *Mus* spp. individuals and two *Rattus* spp. individuals). These were excluded from the results. Another 52 individuals which escaped from traps or during handling were also excluded from the study.

A total of 519 individuals were captured and identified to species level over the course of sampling at the five

sites in the two seasons. These belonged to 13 different species (12 from the Rodentia order and one from the Scandentia order). Of the 519 individuals captured, 395 were captured during the wet season and 124 during the dry season.

*Mus cervicolor*, *Rattus tanezumi* and *Maxomys surifer* were the most dominant species in all sites and seasons (see discussion of the *R. tanezumi*/ R3 clade below), representing 94% and 85% of all individuals captured during the wet and dry season, respectively (Fig. 2). The remaining 10 species were represented by 44 individuals. Species abundance data for each site and season are provided in Table 1.

Community composition differed significantly between seasons (PERMANOVA:  $F=7.13$ ,  $p=0.015$ ), as indicated by a permutational multivariate analysis of variance on a Bray-Curtis dissimilarity matrix. Chao's method (Chiu *et al.*, 2014) was used to estimate the number of unseen species and total species richness for each site in each season (Fig. 3) and indicated this did not differ significantly between provinces ( $p=0.4$ ) or seasons

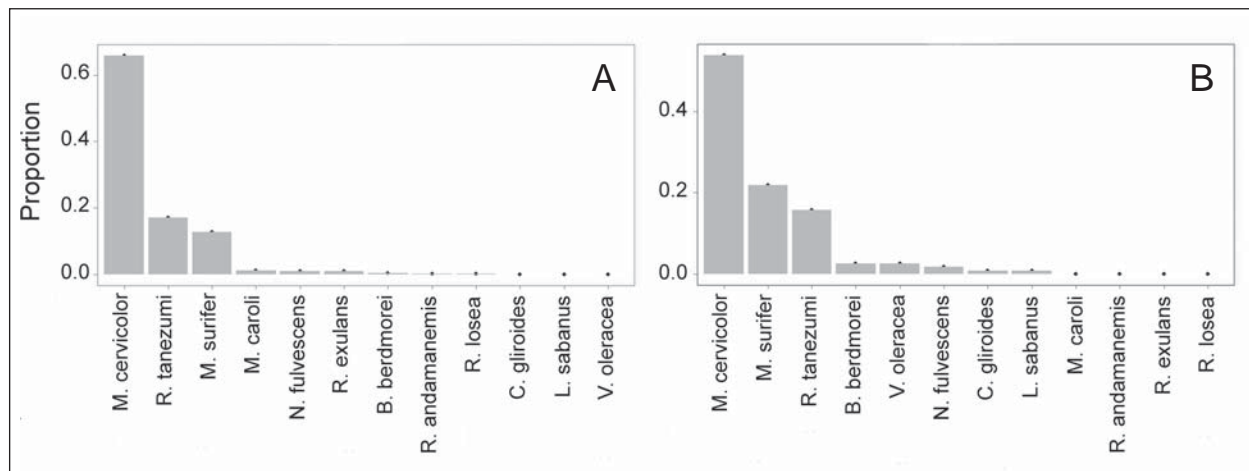
**Table 1** Species captured by site and season in Kampong Thom Province and Mondulhiri Province, 2015–2016.

Species	Season	Mondulhiri			Kampong Thom		Total per season	Total both seasons
		Orona	Pouy Doem Svay	Ochra	Sro Lov Sroung	Rom Chek		
<i>Mus cervicolor</i> (Hodgson, 1845)	Wet	50	45	35	61	64	255	317
	Dry	5	14	1	36	6	62	
<i>Rattus tanezumi</i> <sup>a</sup> (Temminck, 1844)	Wet	14	14	3	23	12	66	84
	Dry	1	3	3	3	8	18	
<i>Maxomys surifer</i> (Miller, 1900)	Wet	8	1	12	6	22	49	74
	Dry	4	5	3	3	10	25	
<i>Tupaia belangeri</i> <sup>b</sup> (Wagner, 1841)	Wet	-	-	-	-	7	7	16
	Dry	1	-	-	3	5	9	
<i>Niviventer fulvescens</i> (Gray, 1867)	Wet	-	1	-	-	4	5	7
	Dry	1	-	-	1	-	2	
<i>Mus caroli</i> (Bonhote, 1902)	Wet	-	-	-	-	5	5	5
	Dry	-	-	-	-	-	-	
<i>Berylmys berdmorei</i> (Blyth, 1851)	Wet	-	1	-	1	-	2	5
	Dry	-	2	-	-	1	3	
<i>Rattus exulans</i> (Peale, 1848)	Wet	-	3	-	1	-	4	4
	Dry	-	-	-	-	-	-	
<i>Vandeleuria oleracea</i> (Bennett, 1832)	Wet	-	-	-	-	-	-	3
	Dry	-	-	-	3	-	3	
<i>Chiropodomys gliroides</i> (Blyth, 1856)	Wet	-	-	-	-	-	-	1
	Dry	-	-	-	-	1	1	
<i>Leopoldamys sabanus</i> (Thomas, 1887)	Wet	-	-	-	-	-	-	1
	Dry	1	-	-	-	-	1	
<i>Rattus losea</i> (Swinhoe, 1870)	Wet	-	-	-	1	-	1	1
	Dry	-	-	-	-	-	-	
<i>Rattus andamanensis</i> (Blyth, 1860)	Wet	1	-	-	-	-	1	1
	Dry	-	-	-	-	-	-	
<b>Total captures</b>	Wet	73	65	50	93	114	395	519
	Dry	13	24	7	49	31	124	
<b>Total rodent species</b> <sup>b</sup>	Wet	4	5	3	6	5	9	12
	Dry	5	4	3	5	5	8	
<b>Chao's estimate of rodent species richness</b> <sup>b</sup>	Wet	4	6	3	9	5	12	14 <sup>c</sup>
	Dry	8	4	3	5	6	10	

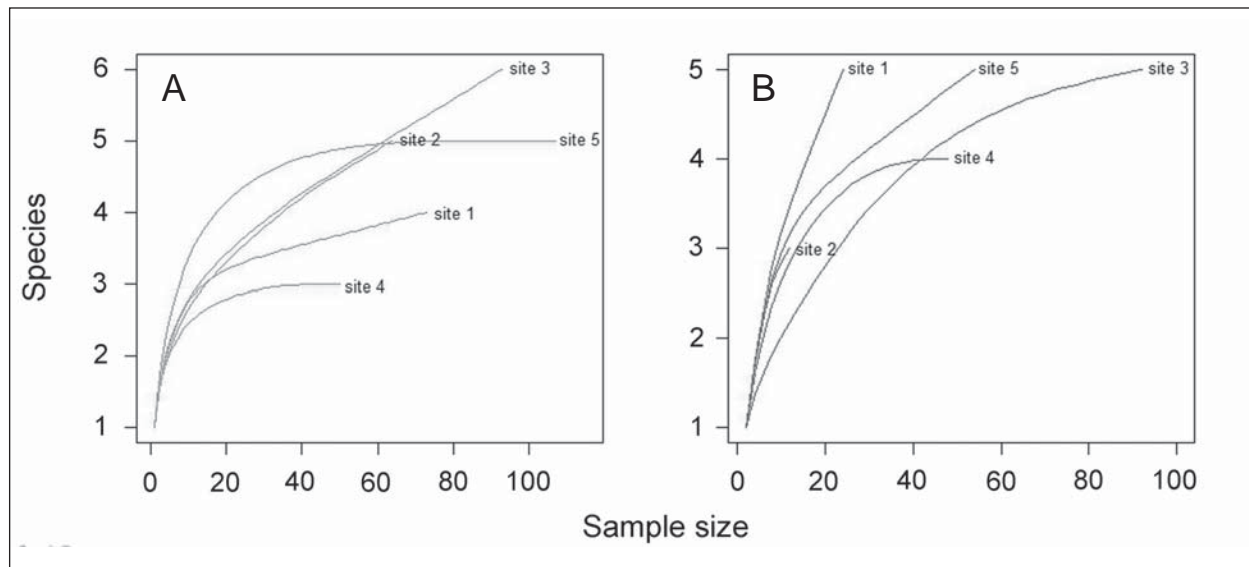
<sup>a</sup> *Sensu* Aplin *et al.* (2011): *Rattus* clade R3. <sup>b</sup> *Tupaia belangeri* is excluded here as it belongs to the Scandentia order (and therefore is not a rodent). <sup>c</sup> Estimated by the bootstrap method.

( $p=0.5$ ). Although the distribution of traps in habitat types followed the same pattern at each site and between seasons, total capture figures for each site were heterogeneous (Table 1). A Poisson regression employing “site” as a random effect indicated that abundance was signifi-

cantly greater during the wet season ( $p<0.0001$ ) and at sites in Kampong Thom Province ( $p<0.0001$ ), suggesting seasonal and large scale geographical variations. Because we could not sample all sites simultaneously, however, the timing of sampling may have confounded our



**Fig. 2** Relative abundance of rodent species in five sites during A) 2015 wet season ( $n=395$ ) and B) 2016 dry season ( $n=124$ ).



**Fig. 3** Rarefaction curves for species richness at five study sites during the A) 2015 wet season and B) 2016 dry season. Site numbers: 1) Orona; 2) Pouy Doem Svay; 3) Sro Lov Sroung; 4) Ochra; 5) Rom Chek.

geographical analysis. Aside from the three dominant species which were recorded at all sites in both seasons, the remaining species were only captured at a few sites.

Ivanova *et al.* (2012) did not observe seasonal variation in rodent abundance in their surveys in Cambodia, but direct comparison of results is difficult due to the greater variety of landscapes and inclusion of village-based rodent captures (with traps provided to villagers) in their study. Climatic variations, and in particular the El Niño Southern Oscillation (ENSO), are known to influence rodent abundance and may have influenced our findings as well as those of previous studies (Ivanova *et al.*, 2012).

The overall species richness of our study (12 Rodentia, 1 Scandantia) is similar to the results of Ivanova *et al.*, (2012), who also found *M. surifer* and *Rattus* spp. were the most dominant species. Unlike other studies in Southeast Asia (Herbreteau *et al.*, 2012; Ivanova *et al.*, 2012; Cosson *et al.*, 2014) however, we did not capture any *Bandicota* spp.

The Asian black rat has a complex evolutionary history which is illustrated by phylogenetic analyses of mitochondrial and nuclear DNA that are incongruent (Aplin *et al.*, 2011; Pagès *et al.*, 2013; Blasdel *et al.*, 2015). To assess lineages of *R. tanezumii*, *R. sakeratensis* and the

**Table 2** Summary characteristics of *Chiropodomys gliroides* and *Vandeleuria oleracea* recorded in Kampong Thom Province during the dry season of 2016.

Site	Location (WGS 84)	Date	Species	Sex	Age	HB <sup>1</sup> (mm)	Tail (mm)
Rom Chek	12.870446 N, 105.487015 E	6 March 2016	<i>C. gliroides</i>	Female	Adult	75.5	103
Sro Lov Sroung	12.853912 N, 105.373021 E	13 February 2016	<i>V. oleracea</i>	Female	Neonate	47.5	74.6
Sro Lov Sroung	12.853879 N, 105.372590 E	17 February 2016	<i>V. oleracea</i>	Female	Juvenile	55.9	87.8
Sro Lov Sroung	12.851962 N, 105.376047 E	18 February 2016	<i>V. oleracea</i>	Female	Neonate	49.5	84.4

<sup>1</sup> HB=Head and body length.

putative *Rattus* taxon known as “R3”, previous studies used diverse genetic markers: two mtDNA genes, one nuclear gene and eight microsatellite loci reflecting different rates of evolution and parental inheritance. Pagès *et al.* (2013) found that *Rattus* sp. R3 was more closely related to *R. tanezumi* on the basis of its nuclear genome (and morphological data) but more closely related to *R. sakeratensis* according to its mitochondrial genome. This was explained by geographical structuring or possible hybridisation with introgression from a species yet to be identified (Pagès *et al.*, 2013; Blasdell *et al.*, 2015). Most of the *R. tanezumi* specimens sampled during our study clustered with the *Rattus* sp. R3 clade (*sensu* Aplin *et al.*, 2011). Growing collections of genetic material for *Rattus* from Cambodia and other parts of Southeast Asia provide opportunities to clarify the taxonomy of the *Rattus* genus.

Two species (*Chiropodomys gliroides* and *Vandeleuria oleracea*) that we captured in Kampong Thom Province are rarely recorded in Cambodia in recent studies (Ivanova *et al.*, 2012; Cosson *et al.*, 2014). The locations of our records lie outside of the geographic ranges provided by IUCN (Lunde *et al.*, 2016; Aplin & Molur, 2017; Fig. 1) and thus represent range extensions. Each individual of these species was identified morphologically (Table 2) because DNA barcode reference sequences are not available for *V. oleracea* and the barcoding approach identified our *C. gliroides* as *M. cervicolor*. Our data will be integrated to the CERoPath database to improve existing DNA barcoding efforts for rodents in Southeast Asia.

In conclusion, our study highlights that rodent populations at our study sites were more abundant during the wet season and dominated by three species (*M. cervicolor*, *R. tanezumi* and *M. surifer*). This structure and dynamic could have significant consequences for the transmis-

sion of important pathogens hosted by rodents, such as *Leptospira*, *Orientia*, *Rickettsia*, hantaviruses and other emerging pathogens (Luis *et al.*, 2013; Morand *et al.*, 2015). Future work will focus on the dynamics of such pathogens in rodent communities and the effects of land-use change at smaller spatial scales.

## Acknowledgements

This study forms part of the One Health LACANET project supported by the European Union (EuropeAid, INNOVATE Programme contract DCI/2013/315-151). The authors are grateful to the field team: Buor Vuthy, Chao Marany, Choerun Unthyda, Hun Udum, In Samat, Kang Sunsisonila, Kuy Lina, Nuon Sithun, Phon Sonara, Ros Sela, San Butum, San Sovannary, Sar Ratha, Sek Seng, Sin Chandara, Suos Sreyem, Ton Sophornn, Torng Sreyleap, Ty Yaren and Y Samnang, and villagers that also helped. We thank Ms Sapho Briand for her skilful project management assistance and Dr Serge Morand for his expert advice. We are also grateful to the Forestry Administration of the Ministry of Agriculture, Forests and Fisheries and to the Ministry of Environment of the Royal Government of Cambodia for authorizing and facilitating our research.

## References

- Aplin, K.P., Suzuki H., Chinen, A.A., Chesser, R.T., ten Have, J., Donnellan, S.C., Austin, J., Frost, A., Gonzalez, J.P., Herbreteau, V., Catzeflis, F., Soubrier, J., Fang Y.P., Robins, J., Matisoo-Smith, E., Bastos, A.D.S., Maryanto, I., Sinaga, M.H., Denys, C., Van Den Bussche, R.A., Conroy, C., Rowe, K., Cooper, A. (2011) Multiple geographic origins of commensalism and complex dispersal history of black rats. *PLoS One*, **6**, e26357.

- Aplin, K. & Molur, S. (2017) *Vandeleuria oleracea*. In *The IUCN Red List of Threatened Species 2017*: e.T22845A22430846. <http://dx.doi.org/10.2305/IUCN.UK.2017-2.RLTS.T22845A22430846>. en [accessed 16 November 2017].
- Blasdell, K., Bordes, F., Chaisiri, K., Chaval, Y., Claude, J., Cosson, J.-F., Latinne, A., Michaux, J., Morand, S., Pagès, M. & Tran A. (2015) Progress on research on rodents and rodent-borne zoonoses in South-east Asia. *Wildlife Research*, **42**, 98.
- Chaval, Y. (2011) *South East Asian Murines Field Guide*. [http://www.ceropath.org/references/rodent\\_field\\_guide](http://www.ceropath.org/references/rodent_field_guide) [accessed 13 November 2017].
- Cosson, J.-F., Picardeau, M., Mielcarek, M., Tatard, C., Chaval, Y., Suputtamongkol, Y., Buchy, P., Jittapalapong, S., Herbreteau, V. & Morand, S. (2014) Epidemiology of *Leptospira* transmitted by rodents in Southeast Asia. *PLoS Neglected Tropical Diseases*, **8**, e2902.
- Francis, C.M. (2008) *A Field Guide to the Mammals of South-East Asia*. New Holland, UK.
- Herbreteau, V., Bordes, F., Jittapalapong, S., Suputtamongkol, Y. & Morand, S. (2012) Rodent-borne diseases in Thailand: targeting rodent carriers and risky habitats. *Infection Ecology & Epidemiology*, **2**, 18637.
- Ivanova, S., Herbreteau, V., Blasdell, K., Chaval, Y., Buchy, P., Guillard, B. & Morand, S. (2012) *Leptospira* and rodents in Cambodia: environmental determinants of infection. *American Journal of Tropical Medicine and Hygiene*, **86**, 1032–1038.
- Luis, A.D., Hayman, D.T.S., O'Shea, T.J., Cryan, P.M., Gilbert, A.T., Pulliam, J.R.C., Mills, J.M., Timonin, M.E., Willis, C.K.R., Cunningham, A.A., Fooks, Rupprecht, C.E., Wood, J.L.N. & Webb, C.T. (2013) A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? *Proceedings of the Royal Society B: Biological Sciences*, **280**, 20122753–20122753.
- Lunde, D., Aplin, K. & Molur, S. (2016) *Chiropodomys gliroides* (errata version published in 2017). In *The IUCN Red List of Threatened Species 2016*: e.T4680A115070127. <http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T4680A22412422>. en [accessed 16 November 2017].
- Meerburg, B.G., Singleton, G.R. & Kijlstra, A. (2009) Rodent-borne diseases and their risks for public health. *Critical Reviews in Microbiology*, **35**, 221–270.
- Morand, S., Bordes, F., Blasdell, K., Pilosof, S., Cornu, J.-F., Chaisiri, K., Chaval, Y., Cosson, J.-F., Claude, J., Feyfant, T., Herbreteau, V., Dupuy, S. & Tran, A. (2015) Assessing the distribution of disease-bearing rodents in human-modified tropical landscapes. *Journal of Applied Ecology*, **52**, 784–794.
- Olival, K.J., Hosseini, P.R., Zambrana-Torrel, C., Ross, N., Bogich, T.L. & Daszak, P. (2017) Host and viral traits predict zoonotic spillover from mammals. *Nature*, **546**, 646–650.
- Pagès, M., Bazin, E., Galan, M., Chaval, Y., Claude, J., Herbreteau, V., Michaux, J., Piry, S., Morand, S. & Cosson, J.-F. (2013). Cytonuclear discordance among Southeast Asian black rats (*Rattus rattus* complex). *Molecular Ecology*, **22**, 1019–1034.
- Singleton, G. (2003) *Impacts of Rodents on Rice Production in Asia*. International Rice Research Institute, Los Baños, Philippines.
- Wilson, D.E. & Reeder, D.M. (2005) *Mammal Species of the World, A Taxonomic and Geographic Reference*. John Hopkins University Press, Baltimore, USA.