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## Rediscovery after thirty years since the last capture of the critically endangered Okinawa spiny rat *Tokudaia muenninki* in the northern part of Okinawa Island

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**Abstract.** The Okinawa spiny rat, *Tokudaia muenninki*, is a critically endangered species endemic to the northern part of Okinawa Island and may be extinct in the wild as there have been no recent sightings of the animal in its natural habitat. We initiated the present search to determine whether the spiny rat still exists in the northern part of Okinawa Island. Sensor cameras and traps were distributed across areas in which past studies had identified the location of occurrence of spiny rats. From a total of 1,276 camera-nights and 2,096 trap-nights from 2007 to 2009, we captured 24 spiny rats; however, we were only successful in identifying spiny rats in the northernmost of the areas sampled, with no indications of the spiny rat in the more southerly areas. The area in which the spiny rats were still present was estimated to be only 1–3 km<sup>2</sup> and is comprised of forest dominated by *Castanopsis sieboldii*, *Lithocarpus edulis*, *Distylium racemosum* and *Schima wallichii*. The trees range in age from about 30 to more than 100 years old, and have an average height of 12 m (range 7 m–16 m). Our rediscovery of the spiny rat in 2008 comes after an interval of 30 years since the previous trapping study in 1978 and seven years since indirect survey evidence from analysis of feral cat feces 2001. Measures for conservation of the location of the spiny rats are urgently required.

**Key words:** body measurements, conservation, critically endangered species, distribution, extinction.

Habitat loss, the impact of invasive species and over-hunting are major factors leading to extinction of threatened mammalian species (Hilton-Taylor 2000). In many cases, species extinction has gone unrecognized due to the lack of continuous monitoring and investigation. Four mammalian species, *Pteropus loochoensis*, *Pipistrellus sturdeeii*, *Canis lupus hattai* and *Canis lupus hodophilax*, have become extinct in Japan and 15 others are critically endangered (Ministry of the Environment 2002). One of these critically endangered species, the Okinawa spiny rat *Tokudaia muenninki* (Rodentia,

Muridae), has not been sighted recently in its natural habitat, raising concerns that it might be extinct in the wild (Kaneko 2005).

The genus *Tokudaia* exists as a relic on the Ryukyu archipelago, the most southwestern region of Japan, and has three species: *T. muenninki* (Johnson 1946) on Okinawa Island, *T. tokunoshimensis* (Endo and Tsuchiya 2006) on Tokunoshima Island and *T. osimensis* (Abe 1933) on Amami-ohshima Island (Endo et al. 2008; Iwasa 2009). The genus is characterized by its possession of an extremely unusual chromosomal constitution

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for mammals. This is exemplified by the different chromosomal constitutions of three species: *T. muenninki* has  $2n = 44$  with an XX/XY sex chromosome system; *T. tokunoshimensis* has  $2n = 45$  with an X0/X0 sex chromosome system; and, *T. osimensis* has  $2n = 25$  with an X0/X0 sex chromosome system (Tsuchiya et al. 1989; Sutou et al. 2001; Arakawa et al. 2002; Kobayashi et al. 2007, 2008; Nakamura et al. 2007; Kuroiwa et al. 2010; Murata et al. 2010). The three species show differences in mtDNA and rDNA sequences, and their cranial characteristics and external measurements also vary (Suzuki et al. 1999, 2000; Kaneko 2001; Endo et al. 2008). The genus *Tokudaia* is thought to be related to *Margaretamys* of Sulawesi or to the fossil *Parapodemus* (Kaneko 2005).

*Tokudaia muenninki* is classified as a critically endangered species, while *T. osimensis* (named *T. tokunoshimensis* before 2006) is designated as endangered in the Japanese Red list (Ministry of the Environment 2002). In 1972, the genus *Tokudaia* was designated a Natural Monument of Japan by the Japanese Government.

The islands in the Ryukyu archipelago show unique biodiversity, including, for example, the Ryukyu long-furred rat *Diplothrix legata* that is endemic to only three islands, Okinawa, Tokunoshima and Amami-ohshima, and the Amami rabbit *Pentalagus furnessi* that is only endemic to two islands, Tokunoshima and Amami-ohshima. *Pentalagus* which is thought to have existed on Okinawa during the Pleistocene as fossil *Pentalagus* were recently discovered there and dated to 1.5–1.7 MYA (Ozawa 2009). These unique species including *Tokudaia* evolved in the absence of terrestrial predatory mammals on these islands.

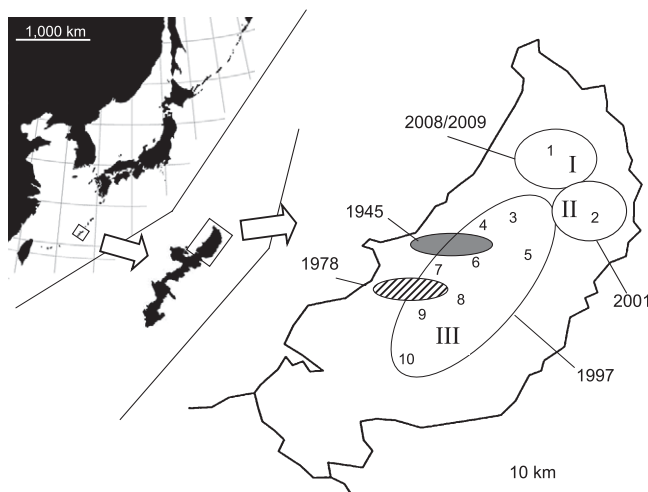
The present study was initiated to determine whether *T. muenninki* was still present in the northern part of Okinawa Island. To this end, we set up sensor cameras and traps to confirm the occurrence of the spiny rats; we also collected tissue samples from trapped animals for cytogenetic comparisons with the other two *Tokudaia* species (Kobayashi et al. 2007, 2008; Nakamura et al. 2007; Kuroiwa et al. 2010; Murata et al. 2010). In this report, we describe the rediscovery of the species and the habitat conditions in the area in which it was still extant. We also obtained body measurement data and determined the reproductive condition of the trapped individuals in order to increase the record on this critically endangered species and to aid its conservation. There is little descriptive information available from live specimens of this species, with the exception of studies in

1978 (Mitsui 1979) and in 1945 (Johnson 1946). We also discuss possible reasons for the reduction in the distribution of this species and consider potential steps to aid its conservation.

## Study areas and methods

### Study areas

The study areas were located in the northern part of Okinawa Island, in which past studies had identified locations in which spiny rats were present (Fig. 1). Three search areas (Areas I, II and III) were investigated in the northern part of Okinawa Island in this study and these are distinguished by geographical location and by the dates on which they were studied. The dates shown for Areas I, II and III, and the ellipse shaded with oblique lines and on the ellipse shaded with black indicate habitats investigated by this and past studies: 2008/2009 in Area I refers to this study; 2001 in Area II was studied by Jogahara et al. (2003); 1997 in Area III was studied by Ohshima et al. (1997); 1978 in Area III was studied



**Fig. 1.** Geographical locations of Areas I, II and III in the northern part of Okinawa Island that were searched for the presence of *Tokudaia muenninki* from 2007 to 2009. For conservation reasons, we show only an approximate outline of the location of the main habitat (Area I). Details on these areas are given in Tables 1, 2 and 6. The date shown on Areas I, II and III, and on the ellipse shaded with oblique lines and on the ellipse shaded with black indicate when these habitats were investigated: 2008/2009 in Area I indicates the present study; 2001 in Area II is the study of Jogahara et al. (2003); 1997 in Area III is the study of Ohshima et al. (1997); 1978 in Area III is the study of Mitsui (1979); 1945 in Area III is the study of Johnson (1946). The main landmarks of these areas are as follows: 1, Mt. Nishime; 2, Mt. Ibu; 3, Mt. Fuenchiji; 4, Yona River; 5, Aha River; 6, Mt. Yonaha; 7, Iji at the Ohkkuni Forest Road; 8, Mt. Iyu; 9, Hiji at the Ohkkuni Forest Road; 10, Mt. Tamatsuji.

by Mitsui (1979); and 1945 in Area III was studied by Johnson (1946) (Fig. 1). The main landmarks of these areas are: 1, Mt. Nishime; 2, Mt. Ibu; 3, Mt. Fuenchiji; 4, Yona River; 5, Aha River; 6, Mt. Yonaha; 7, Iji at the Ohkkuni Forest Road; 8, Mt. Iyu, 9, Hiji at the Ohkuni Forest Road; 10, Mt. Tamatsuji.

The sampled areas consist of broadleaved forests and have valleys that range in elevation from 200 to 300 m above sea level. Annual temperatures varied between 5.2 and 33.9°C, and annual precipitation ranged from 1,978 to 3,603 mm during the ten-year period 1999–2008 at the Oku Observation Point (26°50.0N, 128°16.3E, and 232 m above sea level) in the village of Kunigami. The study areas included 22 forest sites dominated by *Castanopsis sieboldii*.

#### *Sensor camera census*

Between 40 and 60 sets of cameras with the infrared sensors FieldNote I and II (Marif Co., Japan) were used to search for spiny rats from January 2008 to March 2009; in total, nine sites were selected in the study areas (Table 1). At each site monitored by cameras, we set baits consisting of *Castanopsis sieboldii* acorns and peanuts to entice animals into the monitored areas.

#### *Trapping*

Trapping surveys were carried out in March and December 2007, February and March 2008, and February 2009 because of the expectation of high efficiency of capture in the early spring and winter seasons. Between 30 and 140 cage traps, with dimensions of 15 × 10 × 15 cm, were used at 14 sites in the forests. The sites of sensor camera census and trapping surveys did not overlap completely as we did not carry out a sensor camera census in 2007 and we did not carry out trapping surveys if no spiny rats were found in a preliminary camera census. Each trap was covered by a plastic bag to waterproof against rainfall. The traps were baited with several acorns of *Castanopsis sieboldii*, cuts of sweet potato *Ipomoea batatas*, slices of fish sausage, dried small sardines, and peanuts. The traps were set during the daytime and checked thereafter each morning. Spiny rats captured in this study were transported to the Yambaru Wildlife Conservation Center of the Ministry of the Environment in the village of Kunigami where the following measurements were made on unanaesthetized animals held in a mesh bag: body weight (BW), head and body length (HBL), tail length (T), hind foot length excluding the claws (HFL), ear length (EL), and the

length between the anus and the genital organ (Jackson distance). A small piece was cut from the tip of the tail of each spiny rat and used for cytogenetic comparisons with other species. After completion of these procedures, all the spiny rats were released at their capture sites. The Agency for Cultural Affairs (Permission No. 4-2051) and the Ministry of the Environment of Japan (Permission Nos. 080207001, 090120002 and 090601001) permitted capture of a maximum of 5 to 10 individuals per year.

#### *Age classification and sex determination*

We determined the age and sex of each trapped individual to examine the relationship between these factors and the measured external morphological characters (see above). For age classification, females with a body weight greater than 130 g and males heavier than 120 g were classified as adults. This weight classification system is based on the results of a previous study in 1978 that examined the characteristics of sexually mature individuals (Mitsui 1979). Mature females were identified by the presence of an open vaginal orifice and prominent nipples, while the mature males had descended testes. The presence of undeveloped spines on the body was used to identify juveniles. Animals with a lower body weight than an adult but which had fully grown spines were classified as sub-adult.

As externally visible secondary sexual characteristics are only present in adults during the reproductive season and are not visible in juveniles, we used the Jackson distance (see above) to determine the sex of captured individuals. We confirmed this classification by PCR analysis for the *SRY* gene and by karyotype analysis (Murata et al., 2010).

#### *Vegetation in the capture site*

We determined the vegetation profile at each site at which spiny rats were captured. One or three plots (each 10 m<sup>2</sup>) were assessed at each site. Three tree layers and the understory layer, including herbs and ferns, were classified vertically on each plot. A measuring pole was used to determine the heights of trees in the upper tree layer of each plot. The relative plant coverage of each tree layer over the ground was assessed subjectively by eye. Slope inclinations and directions were measured using a clinometer. We assessed a total of nine plots on May 29, 2009 for this study.

The history of forest management at each site where spiny rats were captured was determined based on the

**Table 1.** Number of sensor cameras at each site and the number of cameras which successfully photographed *Tokudaia muenninki* individuals in the northern part of Okinawa Island from 2008 to 2009

Area	Site	Place name*	Period of camera set	Total number of camera-nights	Number of spiny rats photographed	Ratio of spiny rats photographed per 100 camera-nights
I	N6	Mt. Nishime (1)	2008.02.12–05.10	399	9	2.25
I	N11	Mt. Nishime (1)	2009.01.07–25	139	137	98.35
II	Ib1	Mt. Ibu (2)	2008.01.27–02.09	114	0	0.00
III	Y2	Mt. Yonaha (6)	2008.01.13–27	176	0	0.00
III	F1	Mt. Fuenchiji (3)	2008.02.10–14	9	0	0.00
III	O1	Hiji at the Ohkuni Forest Road (9)	2008.02.10–11	5	0	0.00
III	O2	Iji at the Ohkuni Forest Road (7)	2008.04.26–05.16	22	0	0.00
III	YR1	Yona River (4)	2008.12.27–2009. 01.05	91	0	0.00
III	YR1	Yona River (4)	2009.02.27–03.12	322	0	0.00
Mean						11.44
Total				1,276	146	

\* The numbers in parentheses indicate site positions in Fig. 1.

report of Watanabe (2008), who analyzed aerial photos taken in 1970, 1977, 1993 and 2003.

#### Statistical analyses

ANOVA tests with the Holm method were used to analyze differences in external measurements between the females and males collected in this study and between the adult individuals recorded here and those described in a previous studies in 1978 (Mitsui 1979) and in 1945 (Johnson 1946) ; differences were considered significant at  $P < 0.05$ .

## Results

#### Locations of successful captures

The sensor cameras provided of total of 1,276 camera-nights (Table 1). Spiny rats were recognized only in Area I (Fig. 1): the ratio of photo captures of spiny rats was highest at Site N11 in Area I with 98.35 photos /100 camera-nights; the ratio of photo captures was lower (2.25/100 camera-nights) at Site N6 in Area I (Table 1). The sensor cameras did not identify spiny rats in Areas II and III (Table 1).

In the trapping survey, a total of 2,096 trap-nights was used (Table 2). Spiny rats were only captured in Area I. The capture ratio at Site N11 in Area I was highest at 24.14 spiny rats per 100 trap-nights (Table 2). Significant ratios of trapping success were also obtained for Sites N7 (7.81), N6 (3.94), and N9 (2.22) in Area I, and low ratios in three sites (N8, N3 and N4) (Table 2).

However, even in Area I, no spiny rats were captured at Sites N1, N2, N5, or N10.

The estimated sizes of the trapping sites were 0.06 km<sup>2</sup> for Site N3 and N4, 0.12 km<sup>2</sup> for Sites N6, N7, N8 and N9, and 0.005 km<sup>2</sup> for Site N11. Therefore, the main area where spiny rats occurred, estimated as the polygon from the most external points of each captured site, was 1–3 km<sup>2</sup> in Area I (Fig. 1). For reasons of conservation, we have presented only a rough outline of this region in Figure 1.

No spiny rats were recorded in Areas II and III using photo census or trapping methods.

#### Vegetation profile of the capture site

The topography of the main site in which spiny rats were found included small ridges, slopes and small mountain valleys. Overall, the topographies of the sampling Areas did not vary significantly, and showed a mean slope of 17° (range 5–25°) with variable direction (Table 3). The average tree height in main site was 12 m (range 7 m–16 m), and the trees ranged in age from about 30 years to more than 100 years old. The relative plant coverage over the ground in the upper tree layer was approximately 71% (40–90%), approximately 71% (30–90%) in the middle tree layer, approximately 66% (30–100%) in the lower tree layer, and approximately 22% (5–40%) in the understory layer (Table 3). The capture sites of the spiny rats were in forest dominated by *Castanopsis sieboldii*, *Lithocarpus edulis*, *Distylium racemosum* and *Schima wallichii* in the upper tree layer,

**Table 2.** Number of traps and number of *Tokudaia muenninki* individuals captured in the northern part of Okinawa Island from 2007 to 2009

Area	Site	Place name*	Period of traps set	Total number of nights	Total number of trap-nights	Number of spiny rats captured	Capture ratio of spiny rats per 100 trap-nights
I	N1	Mt. Nishime (1)	2007.03.06–09, 20–22	7	59	0	0.00
I	N2	Mt. Nishime (1)	2007.12.24–29	6	67	0	0.00
I	N3	Mt. Nishime (1)	2008.02.28–03.05	6	326	4	1.23
I	N4	Mt. Nishime (1)	2008.03.03–07	5	150	1	0.67
I	N5	Mt. Nishime (1)	2008.03.09–10	2	18	0	0.00
I	N6	Mt. Nishime (1)	2009.02.14–17	4	127	5	3.94
I	N7	Mt. Nishime (1)	2009.02.14–17	4	64	5	7.81
I	N8	Mt. Nishime (1)	2009.02.14–17	4	76	1	1.32
I	N9	Mt. Nishime (1)	2009.02.15–17	3	45	1	2.22
I	N10	Mt. Nishime (1)	2009.02.15–17	3	36	0	0.00
I	N11	Mt. Nishime (1)	2009.02.16–17	2	29	7**	24.14
II	Iy1	Mt. Iyu (8)	2007.03.06–11	6	66	0	0.00
II	Iy1	Mt. Iyu (8)	2007.12.24–29	6	44	0	0.00
II	Ib1	Mt. Ibu (2)	2008.02.15–24	10	923	0	0.00
III	Y1	Mt. Yonaha (6)	2007.03.15–20	6	66	0	0.00
Mean							1.15
Total				74	2,096	24	

\* The numbers in parentheses indicate site positions in Fig. 1.

\*\* Seven spiny rats captured at this site were released without measurement because they were in excess of the number permitted by the protection laws.

by *Styrax japonica*, *Camellia japonica* and *Schefflera octophylla* in the middle layer, by *Pleioblastus linearis* and *Ardisia quinquegona* in the lower layer, and by *Alpinia intermedia*, *Cyathea podophylla* and *Blechnum orientale* in the understory layer. Acorns from *Castanopsis sieboldii* and *Lithocarpus edulis* trees, a principal food source of spiny rats in winter, had already disappeared from the ground at the time of the investigation (May 29, 2009).

Area I contained many more areas of unlogged forest compared to Areas II and III; according to a previous report, no logging had ever occurred at capture sites N3, N4, N6, N7 or N8 in Area I (Table 3) (Watanabe 2008). However, Area I had suffered deforestation at site N9 about 30 years ago, and deforestation and burning, prior to the establishment of *Pinus luchuensis* plantations, had occurred at site N11 about 30 years ago.

#### Body measurements of spiny rats captured in this study

In this study, five spiny rats were captured on March 3 and 9 in 2008 and 19 animals were captured on February 16 and 17 in 2009 in the most successful sites of Area I (Table 4). External measurements were made, reproductive status was examined, and tails tips were collected for cytogenetic analysis from 17 of the 24 spiny rats. The remaining seven animals were returned to Area I in 2009

without any measurements or tissue collection because they were in excess of the permitted number. All of the other spiny rats were subsequently released at their respective capture sites except for individual No. 4 (Table 4), which was almost dead in the trap when found due to being bitten by a dwarf lance head snake *Ovophis okinavensis* that had entered the trap first.

The body weights of the nine females measured in this study ranged from 51.0 to 169.0 g, and those of the eight males from 43.4 to 154.2 g (Table 4). The sample of females included two juveniles (11.8% of total 17 spiny rats), two sub-adults (11.8%) and five adults (29.4%); the male group included two juveniles (11.8%), two sub-adults (11.8%) and four adults (23.5%) (Table 4). The sex ratio (male/total) for the spiny rats in this study was 0.5, 0.5, 0.4 and 0.5 for juvenile, sub-adult, adult and total individuals, respectively.

No significant inter-sex differences between adult females and adult males were found for the measured external characters, except for the Jackson distance and the ratio of the Jackson distance to HBL in the individuals collected in this study (Table 5). In adult spiny rats, the mean Jackson distance in males (mean  $\pm$  SE: 27.0 mm  $\pm$  1.91,  $n = 4$ ) was significantly larger than that of females (16.5 mm  $\pm$  1.95,  $n = 4$ ) ( $P = 0.008$ ,  $F = 14.795$ ) (Table 5). The mean ratio of the Jackson distance to

Table 3. Vegetation profiles of each location where spiny rats were captured in Area I in this study

Sites	Individual no. of spiny rats*	Inclination (°)	Direction of slope	Height of tree (m)	% of plant coverage over the ground in each tree layer				Plant species in each tree layer			History of forest management based on Watanabe (2008)	Forest age in 2009		
					Upper	Middle	Lower	Understory	Upper**	Middle	Lower			Understory	
N3	6	15	S40W	11	90	70	50	20	<i>Castanopsis sieboldii</i> <i>Lithocarpus edulis</i> <i>Schima wallichii</i>	<i>Ilex goshiensis</i> <i>Distylium racemosum</i> <i>Schefflera octophylla</i>	<i>Ardisia quinquegona</i> <i>Camellia lutchuensis</i>	<i>Alphimia intermedia</i> <i>Cyathea podophylla</i>	No cutting post-1970	Old	
N4	9	5	N50W	8	50	70	90	20	<i>Castanopsis sieboldii</i> <i>Myrica rubra</i>	<i>Elaeocarpus japonicus</i> <i>Rhaphiolepis indica</i>	<i>Syzygium buxifolium</i> <i>Psychotria rubra</i>	<i>Alphimia intermedia</i> <i>Blechnum orientale</i> <i>Cyathea podophylla</i>	No cutting post-1970	Old	
N6	13	15	N60E	13	40	70	100	30	<i>Distylium racemosum</i> (150–200 years old) <i>Castanopsis sieboldii</i> (ca. 100 years old) <i>Meliosma rigida</i>	<i>Camellia lutchuensis</i> <i>Elaeocarpus japonicus</i> <i>Daphniphyllum teijsmannii</i>	<i>Neolitsea sericea</i> <i>Ardisia quinquegona</i> <i>Pleioblastus linearis</i>	<i>Alphimia intermedia</i> <i>Cyathea podophylla</i>	No cutting post-1970	Old	
N7	2	15	N60E	7	90	90	80	20	<i>Lithocarpus edulis</i> <i>Castanopsis sieboldii</i>	<i>Cinnamomum sieboldii</i> <i>Syrax japonica</i> <i>Dendropanax trifidus</i>	<i>Castanopsis sieboldii</i> <i>Pleioblastus linearis</i>	<i>Alphimia intermedia</i>	No cutting post-1970	Middle	
N8	14	15	N80E	10	90	80	90	20	<i>Castanopsis sieboldii</i> <i>Lithocarpus edulis</i>	<i>Schefflera octophylla</i> <i>Elaeocarpus japonicus</i>	<i>Pleioblastus linearis</i> <i>Disyllum racemosum</i> <i>Syzygium buxifolium</i>	<i>Alphimia intermedia</i>	No cutting post-1970	Middle	
N9	15	20	N280W	10	70	90	50	5	<i>Lithocarpus edulis</i> (ca. 40 years old) <i>Castanopsis sieboldii</i>	<i>Dendropanax trifidus</i> <i>Camellia japonica</i> <i>Distylium racemosum</i>	<i>Pleioblastus linearis</i> <i>Antidesma japonicum</i>	<i>Alphimia intermedia</i>	Cut in 1977	Middle	
N11	–	25	N270W	16	50	70	70	20	<i>Pinus lutchuensis</i> (ca. 30 years old) <i>Castanopsis sieboldii</i>	<i>Syrax japonica</i> <i>Schima wallichii</i> <i>Schefflera octophylla</i> <i>Tutcheria virgata</i>	<i>Pleioblastus linearis</i> <i>Diospyros morrisiana</i> <i>Psychotria rubra</i>	<i>Cyathea podophylla</i>	<i>Pinus</i> seedling plantation after cutting and burning in 1977	Late young	
N11	–	15	N270W	16	70	70	30	40	<i>Castanopsis sieboldii</i> <i>Pinus lutchuensis</i> (plantation) <i>Schima wallichii</i>	<i>Syrax japonica</i> <i>Wendlandia formosana</i>	<i>Dendropanax trifidus</i> <i>Ardisia quinquegona</i> <i>Schefflera octophylla</i>	<i>Alphimia intermedia</i> <i>Cyathea podophylla</i>	<i>Pinus</i> seedling plantation after cutting and burning in 1977	Late young	
N11	–	10	N20W	14	90	30	30	20	<i>Cyathea lepidifera</i> <i>Pinus lutchuensis</i> (plantation) <i>Lithocarpus edulis</i> <i>Syrax japonica</i>	<i>Castanopsis sieboldii</i> <i>Schefflera octophylla</i> <i>Lithocarpus edulis</i>	<i>Wendlandia formosana</i> <i>Meliosma rigida</i>	<i>Alphimia intermedia</i> <i>Cyathea podophylla</i> <i>Blechnum orientale</i>	<i>Pinus</i> seedling plantation after cutting and burning in 1977	Late young	
Means		17		12	71	71	66	22							

\* see also, Table 4; –, not measured.

\*\* parenthesis indicate trees in age estimated by observation in the field.

**Table 4.** External measurements of 17 *Tokudata muenninki* individuals captured in Area I in the northern part of Okinawa Island in 2008 and 2009

Individual no. of spiny rat	Sex*	Age class	Body weight (g) (BW)	Head and body length (mm) (HBL)	Hind foot length excluding claw (mm) (HFL)	Ear length (mm) (EL)	Tail length (mm) (T)	Ratio of tail length (%) (T/HBL × 100)	Distance between anus and genital organ (mm) (Jackson distance)	Ratio of Jackson distance (%) (Jackson distance/HBL × 100)	Date of capture	Remarks
1	female	juvenile	51.0	97	28.5	20.8	96	98.9	9.4	9.7	2008.03.09	Vaginal orifice closed, spines on body obscure
2	female	juvenile	52.9	88	28.8	12.4	94	106.8	7.7	8.8	2009.02.17	Vaginal orifice closed, spines on body obscure
3	female	sub-adult	75.2	95	31.7	17.0	107	112.6	15.0	15.8	2009.02.17	Vaginal orifice closed, spines on body obvious
4	female	sub-adult	94.0	116	31.7	16.0	113	97.4	11.5	9.9	2008.03.09	Vaginal orifice closed, spines on body obvious
5	female**	adult	132.0	128	32.4	20.4	123	96.1	21.9	17.1	2009.02.17	Vaginal orifice closed, 2 pairs of prominent nipples, spines on body obvious
6	female	adult	139.0	130	32.3	18.2	105	80.4	—	—	2008.03.03	Vaginal orifice closed, 2 pairs of prominent nipples, spines on body obvious
7	female	adult	139.2	140	32.7	19.7	115	82.1	14.5	10.4	2009.02.17	Vaginal orifice closed, 2 pairs of prominent nipples, spines on body obvious
8	female	adult	141.0	121	32.4	15.9	116	95.9	13.0	10.7	2009.02.17	Trace vaginal orifice present, 2 pairs of prominent nipples, spines on body obvious
9	female	adult	169.0	167	34.3	18.8	121	72.5	16.6	9.9	2008.03.09	Vaginal orifice closed, 2 pair of nipple prominent, spines on body obvious
10	male	juvenile	43.4	94	30.0	14.9	90	95.7	15.0	16.0	2009.02.16	No testis descended, spines on body obscure
11	male**	juvenile	67.7	100	30.9	14.8	99	99.0	11.0	11.0	2009.02.16	No testis descended, spines on body obscure
12	male**	sub-adult	93.2	104	33.8	13.4	106	101.9	16.0	15.4	2009.02.17	No testis descended, spines on body obvious
13	male	sub-adult	95.3	130	32.4	15.6	101	77.7	19.3	14.8	2009.02.16	No testis descended, spines on body obvious
14	male	adult	122.4	128	33.6	19.9	124	96.9	28.0	21.9	2009.02.17	No testis descended, spines on body obvious
15	male	adult	144.0	145	33.8	17.0	120	82.8	24.0	16.6	2008.03.09	No testis descended, spines on body obvious
16	male	adult	144.3	150	32.5	17.1	128	85.3	24.0	16.0	2009.02.17	No testis descended, spines on body obvious
17	male	adult	154.2	140	34.4	20.3	135	96.4	32.0	22.9	2009.02.17	No testis descended, spines on body obvious

In 24 spiny rats captured in this study, 17 spiny rats were measured and the remaining seven spiny rats captured in 2009 were released without measurement because they were in excess of the permitted number.

\* sex confirmed by PCR analysis for the *Sry* gene and by karyotype analysis (Murata et al. 2010); \*\* wrong sex determined by the Jackson distance.

For age classification, females with a body weight greater than 130 g and males heavier than 120 g were classified as adults; this classification is based on characteristics of sexual maturity from a previous study in 1978 (Mitsui 1979).

—, not measured.



Table 5. Comparison of mean values of external measurements of adult *Tokudaia mueminki* collected in 2008/2009, 1978 and 1945

Sex	Statistics	Body weight (g) (BW)	Head and body length (mm) (HBL)	Hind foot length excluding claw (mm) (HFL)	Ear length (mm) (EL)	Tail length (mm) (T)	Ratio of tail length (T/HBL × 100) (%)	Distance between anus and genital organ (mm) (Jackson distance)	Ratio of Jackson distance (Jackson distance/HBL × 100) (%)	Reference
Female	<i>n</i>	5	5	5	5	5	5	4	4	
	<i>mean</i>	144.0	137	32.8 <sup>i</sup>	18.7 <sup>ii</sup>	111	85.4 <sup>iii</sup>	16.5 <sup>iv</sup>	12.0 <sup>v</sup>	
	<i>SE</i>	6.43	8.3	0.38	0.82	4.6	4.62	1.95	1.7	
	<i>range</i>	132.0–169.0	121–167	32.3–34.3	15.9–20.8	96–121	72.5–96.1	13.0–21.9	9.9–17.1	
Male	<i>n</i>	4	4	4	4	4	4	4	4	This study (Individuals collected in February 2008 and May 2009)
	<i>mean</i>	141.2	141	33.5	18.6 <sup>iii</sup>	127 <sup>vi</sup>	90.3 <sup>vii</sup>	27.0 <sup>ix</sup>	19.4 <sup>x</sup>	
	<i>SE</i>	6.70	4.7	0.4	0.9	3.2	3.7	1.91	1.8	
	<i>range</i>	122.4–154.2	128–150	32.5–34.4	17.0–20.3	120–135	82.8–96.9	24.0–32.0	16.0–22.9	
Total	<i>n</i>	9	9	9	9	9	9	–	–	
	<i>mean</i>	142.8	139 <sup>ab</sup>	33.2 <sup>c</sup>	18.6 <sup>d</sup>	118	87.6 <sup>de</sup>	–	–	
	<i>SE</i>	4.4	4.7	0.3	0.6	4.0	3.0	–	–	
	<i>range</i>	122.4–169.0	121–167	32.3–34.4	15.9–20.8	97–135	72.5–96.9	–	–	
Female	<i>n</i>	5	5	5	3	5	5	–	–	Mitsui (1979) (Individuals collected in May–September 1978)
	<i>mean</i>	168	153	35	17 <sup>iv</sup>	116	76	–	–	
	<i>SE</i>	9.85	3.5	0.55	2.33	4.4	1.31	–	–	
	<i>range</i>	148–200	147–166	33–36	15–22	106–132	71.6–79.5	–	–	
Male	<i>n</i>	6	6	6	6	6	6	–	–	
	<i>mean</i>	142	153	34	19 <sup>v</sup>	119	78	–	–	
	<i>SE</i>	9.53	5.1	0.9	1.0	2.9	3.6	–	–	
	<i>range</i>	123–186	140–176	31–37	16–22	110–127	69.3–90.0	–	–	
Total	<i>n</i>	11	11	11	9	11	11	–	–	
	<i>mean</i>	154	153 <sup>a</sup>	34	19 <sup>c</sup>	118	77 <sup>f</sup>	–	–	
	<i>SE</i>	7.7	3.1	0.5	1.0	2.5	2.0	–	–	
	<i>range</i>	123–200	140–176	31–37	15–22	106–132	69.3–90.0	–	–	
Female	<i>n</i>	–	5	(5)	3	5	5	–	–	Johnson (1946) (Individuals collected in September 1945)
	<i>mean</i>	–	155	(35)	27 <sup>iiii,v,vv</sup>	108 <sup>vi</sup>	70 <sup>viii</sup>	–	–	
	<i>SE</i>	–	4.8	(0.7)	3.5	2.9	1.2	–	–	
	<i>range</i>	–	141–166	(34–38)	23–34	99–117	65–72	–	–	
Male	<i>n</i>	–	6	(6)	5	5	5	–	–	
	<i>mean</i>	–	154	(36) <sup>i</sup>	24	113	76	–	–	
	<i>SE</i>	–	4.7	(0.4)	0.5	2.5	2.1	–	–	
	<i>range</i>	–	144–175	(34–37)	22–25	104–119	70–80	–	–	
Total	<i>n</i>	–	11	(11)	8	10	10	–	–	
	<i>mean</i>	–	154 <sup>b</sup>	(36) <sup>c</sup>	25 <sup>de</sup>	111	73 <sup>g</sup>	–	–	
	<i>SE</i>	–	3.2	(0.4)	1.3	2.0	1.5	–	–	
	<i>range</i>	–	141–175	(34–38)	22–34	99–119	65.1–80.0	–	–	

For age classification in this study and Mitsui (1979), females with a body weight greater than 130 g and males heavier than 120 g were classified as adults; this classification is based on characteristics of sexual maturity from a previous study in 1978 (Mitsui 1979).

→, not measured.

Parenteses in HFL in Johnson (1946) mean the values measured with claw.

i,  $P = 0.003$ ;  $F = 3.79$ ; ii,  $P = 0.004$ ;  $F = 6.029$ ; iii,  $P = 0.004$ ;  $F = 6.029$ ; iv,  $P = 0.033$ ;  $F = 6.029$ ; v,  $P = 0.004$ ;  $F = 6.029$ ; vi,  $P = 0.004$ ;  $F = 3.315$ ; vii,  $P = 0.004$ ;  $F = 5.358$ ; viii,  $P = 0.003$ ;  $F = 5.358$ ; ix,  $P = 0.008$ ;  $F = 14.795$ ; x,  $P = 0.025$ ;  $F = 8.874$ .

a,  $P = 0.017$ ;  $F = 5.24$ ; b,  $P = 0.025$ ;  $F = 5.24$ ; c,  $P = 0.017$ ;  $F = 8.37$ ; d,  $P = 0.017$ ;  $F = 12.87$ ; e,  $P = 0.025$ ;  $F = 12.87$ ; f,  $P = 0.017$ ;  $F = 11.37$ ; g,  $P = 0.025$ ;  $F = 11.37$ .

**Table 6.** Records of distribution of the spiny rat in Areas I, II and III in the northern part of Okinawa Island from 1945 to 2009

Year of investigation	Area of investigation	Areas inhabited by spiny rats	Method of confirmation	Number of fecal samples that included spines/number of samples analyzed	Frequency of spines per sample (%)	Reference
2008–2009	I, II, III	I	Sensor camera and trapping	–	–	This study
2001–2009	I, II, III	I	Mongoose trapping	–	–	Ministry of the Environment (2009)
2001	II, III	II	Cat feces analysis	1/28	3.6	Jogahara et al. (2003)
1999	I, II	–	Cat, dog and mongoose feces analysis	0/22	0.0	Kawauchi and Sasaki (2002)
1997	III	III	Cat feces analysis	7/26	12.5	Ohshima et al. (1997)
1981	III	III	Cat feces analysis	16/20	80.0	Okinawa Prefectural Board of Education (1981)
1978	I, III	III	Trapping	–	–	Mitsui (1979)
1975	II, III	II, III	Cat feces analysis	9/12	75.0	Miyagi (1976)
1945	III	III	Trapping	–	–	Johnson (1946)

–, not analysed.

HBL in adult males ( $19.4\% \pm 1.8$ ,  $n = 4$ ) was significantly larger than in adult females ( $12.0\% \pm 1.7$ ,  $n = 4$ ) ( $P = 0.025$ ,  $F = 8.874$ ) (Table 5). In young spiny rats, the mean Jackson distances in males (13.0 mm in juvenile and 17.7 mm in sub-adult) were larger than those in females (8.5 mm in juvenile and 13.3 mm in sub-adult) with no significant differences (Table 4). The mean ratios of the Jackson distance to HBL in males (13.5% in juvenile and 15.1% in sub-adult) were larger than those in females (9.3% in juvenile and 12.9% in sub-adult) although the differences were not significant (Table 4). PCR based sex determination identified three individuals (17.6% of the total of 17 individuals) that appeared to have been assigned to the wrong sex on the basis of their Jackson distances (see Table 4): animal No. 5 in adult (female by PCR, male by Jackson distance of 21.9 mm), animal No. 11 in juvenile (male by PCR, female by Jackson distance of 11.0 mm) and animal No. 12 in sub-adult (male by PCR, female by Jackson distance of 16.0 mm).

With regard to reproductive condition, the vaginal orifice was closed and two pairs of nipples were prominent in the four adult females (Nos. 5, 6, 7 and 9) collected on March 3 and 9, 2008 and February 17, 2009 (Table 4). There was trace evidence of a vaginal orifice in a single adult female (No. 8) collected on February 17, 2009. None of the four adult males (Nos. 14, 15, 16 and 17) collected on March 9, 2008 and February 17, 2009 had

descended testes. The juvenile females and males did not show clear body spines.

There were no significant inter-sex differences in the five external measurements (HBL, HFL, EL, T and T/HBL) between adult females and adult males within each three groups collected in this study and in the past two studies in 1978 (Mitsui 1979) and 1945 (Johnson 1946), and there were no significant inter-sex differences in the body weight within each two groups collected in this study and in 1978 (Mitsui 1979) (Table 5). Therefore, we compared the body measurement of individuals including both sexes in each group. The mean HBL in total individuals including both sexes in this study ( $139 \pm 4.7$  mm,  $n = 9$ ) was significantly shorter than that in 1978 ( $153 \pm 3.1$  mm,  $n = 11$ ) ( $P = 0.017$ ,  $F = 5.24$ ) and that in 1945 ( $154 \pm 3.2$  mm,  $n = 11$ ) ( $P = 0.025$ ,  $F = 5.24$ ), but there were no significant differences in mean tail length among three groups (Table 5). Therefore, the mean ratio of tail length in this study ( $87.6 \pm 3.0\%$ ,  $n = 9$ ) was significantly greater than that in 1978 ( $77 \pm 2.0\%$ ,  $n = 11$ ) ( $P = 0.017$ ,  $F = 11.37$ ) and that in 1945 ( $73 \pm 1.5\%$ ,  $n = 10$ ) ( $P = 0.025$ ,  $F = 11.37$ ). The mean EL in 1945 ( $25 \pm 1.3$  mm,  $n = 8$ ) was significantly longer than that in 1978 ( $19 \pm 1.0$  mm,  $n = 9$ ) ( $P = 0.025$ ,  $F = 12.87$ ) and that in this study ( $18.6 \pm 0.6$  mm,  $n = 9$ ) ( $P = 0.017$ ,  $F = 12.87$ ). The mean HFL in this study ( $33.2 \pm 0.3$  mm,  $n = 9$ ) was significantly shorter than that in 1945 ( $36 \pm 0.4$  mm,  $n = 11$ ) ( $P = 0.017$ ,  $F = 8.37$ ) because the values of HFL by

Johnson (1946) were measured including claw. There were eight significant differences in the mean values of the four external measurements (HFL, EL, T and T/HBL) among each sex group in three collections (see Table 5). Although the mean body weights did not differ significantly between individuals collected in this study and in 1978 (Mitsui 1979) (Table 5), the heaviest individuals recorded in this study (169 g for female and 154 g for male) were lighter than those recorded in 1978 (200 g for female and 186 g for male).

## Discussion

### *Reduction in distribution of spiny rats and their rediscovery*

Our study provides the first evidence of the current range and distribution of the spiny rat on Okinawa Island. There have been few previous reports on the distribution of the spiny rat, and the available information is derived from two trapping investigations and from analyses of the feces of invasive predators, such as feral cats *Felis silvestris catus*, dogs *Canis lupus familiaris* and mongoose *Herpestes auro-punctatus* (Table 6). We used *H. auro-punctatus* here instead of *H. javanicus* as the scientific name of the mongoose based on the recent molecular phylogenetical studies (Veron et al. 2007; Gilchrist et al. 2009; Patou et al. 2009).

In regions to the south of the main location (Area I) identified in this study, there is some evidence of the occurrence of spiny rats in Areas II and III in the 7 to 11 years prior to our rediscovery of spiny rats in 2008. Remnants of spiny hairs from spiny rats were identified in cat feces from Ohgimi Village on Mount Tamatsuji in 1997 (No. 10 in Fig. 1), which is thought to be the most southern habitat of the species (Ohshima et al. 1997). They were also identified in cat feces from Mount Yonaha in 1975–1997 (No. 6) (Miyagi 1976; Okinawa Prefectural Board of Education 1981; Ohshima et al. 1997), from the headwaters of the Aha River in 1997 (No. 5) (Ohshima et al. 1997) and from the Fun River in 2001 (No. 2) (Jogahara et al. 2003). The frequency of spiny rat remains in cat feces decreased from 75.0% in 1975 and 80.0% in 1981 to 3.6% in 2001 in Areas II and III (Table 6). The available information indicates that the distribution of spiny rats has declined during 1975–2001.

There are no data on the hunting ranges of feral cats in northern Okinawa Island, thus, the distance between the place where a cat fed on a spiny rat and the place where it

defecated is unknown. Studies on feral cats elsewhere indicate that they do not travel long distances between their sites of feeding and their defecation places in a single day, depending on food conditions and their home range (Fitzgerald and Turner 2000). Feral cats average 519 m per day (range 30–1,770 m) for hunting, and defecate one scat per day (Konecny 1987; Fitzgerald and Turner 2000). Therefore, it is likely that the information on spiny rat distribution obtained from analysis of feral cat feces provides a reasonably accurate indication of past distribution patterns.

The negative impact of predation by invasive mammals, as described above, may have accelerated extinction of the spiny rat in the northern part of Okinawa Island. In addition, loss of suitable habitats may also have affected spiny rat as logging occurred in Areas II and III between 1970 and 1990 (Watanabe 2008). In contrast, large-scale logging has not occurred in Area I (Table 3) (Watanabe 2008). Therefore, the negative impacts for spiny rats in Areas II and III may largely be a consequence of a combination of cat predation since the 1970s and habitat loss from logging. Subsequent to the study in 2001 (Jogahara et al. 2003), no evidence of spiny rat remains has been obtained from analyses of cat feces or those of other predators; this led to the suggestion that the spiny rat might be extinct on Okinawa Island.

Contrary to the suggestion of species extinction, our sensor camera and trapping analyses of Area I identified an extant of spiny rats. Although we also examined potential habitats for spiny rats in Areas II and III during 2007 and 2009, we obtained no evidence for their occurrence in these areas. We therefore suspect that the population in Area I might contain the only significant group of spiny rats in the northern part of Okinawa Island. In addition, the mongoose capture project in northern part of Okinawa Island (including Areas I, II and III) from 2001 to 2009, with a total of more than 3,000,000 trap-days, did not trap any spiny rats except a few spiny rats trapped in the same location as in the study in Area I (Table 6) (Ministry of the Environment 2009). Our rediscovery of spiny rats in the northern part of Okinawa Island and identification of their current main region of occurrence in this study comes after an interval of 30 years since the previous trapping study in 1978 (Mitsui 1979) and of seven years since survey evidence in 2001 (Jogahara et al. 2003).

The disappearance of spiny rats from the southern part of their previous distribution range area and the reduc-

tion in their numbers on Okinawa Island is similar to that recorded for the Okinawa rail *Gallirallus okinawae*. In 2001, the southern limit of the Okinawa rail was about 10 km further north compared to 1986 and a central area of occurrence had also disappeared, probably due to the negative impacts of invasive predators, such as the mongoose and feral cat (Ozaki et al. 2002; Yamada et al. 2009).

#### *Characteristics of external measurements and reproduction*

There were no significant difference in external measurements between adult females and males in this study, except for the Jackson distance and the ratio of the Jackson distance to HBL. These latter characteristics, especially the Jackson distance, are useful for determining the sex of individuals during the non-breeding season, and also in juveniles and sub-adults. However, our PCR analysis suggested the possibility of a low rate of errors in distinguishing the sexes based only on the Jackson distance. The errors are likely to result from inter-individual variation or incorrect measurements, and it will be necessary to carry out further PCR analyses on a larger number of samples to determine the cause.

The mean HBL in this study ( $139 \pm 4.7$  mm) was significantly shorter than the previous two groups collected in 1978 (Mitsui 1979) ( $153 \pm 3.1$  mm) and 1945 (Johnson 1946) ( $154 \pm 3.2$  mm). And the maximum body weights (169 g for females and 154 g for males) in this study were lower than those (200 g for females and 186 g for males) from animals collected during the non-breeding season (May to September) in 1978 (Mitsui 1979), but there was no significant difference in the mean body weight between two groups (Table 5). Although our sample size is small, the apparent reduction in large-sized individuals in the present study is likely due to deterioration in food availability, reduction of habitat size, and reduction of population. Further studies are necessary to determine and monitor the vulnerability of the population.

With regard to reproduction, juvenile and sub-adult individuals were collected in this study from mid-February to early March. The adult individuals collected here displayed no indications of reproductive activity. We conclude, therefore, that the period from mid-February to early March is part of the non-breeding season and is the time when sub-adults and juveniles switch from being nursed to independence. The breeding season (parturition) is thought to be during October and November, with a litter size of 5–10 (Kaneko 2005).

Characterization of external aspects of the body morphology of the spiny rat is of value for distinguishing this species from the invasive black rat *Rattus rattus*, which lives allopatrically on Okinawa Island. The relative length of the tail to HBL (T/HBL %) in the spiny rat was 87.6% for adults (85.4% for females and 90.3% for males; Table 5) in the study, which is substantially lower than that of the black rat (109.3%; Yoshida et al. 1971). Thus, in a field survey with an auto sensor camera, any individual observed with such characteristics as a relatively short and slender tail, a dark body color and a prominent forehead is likely to be a spiny rat.

#### *Conservation*

Measures for conservation of the main region in Area I where the spiny rats are located are urgently required. In particular, measures against the negative impact of habitat degradation due to logging and of invasive species, such as feral cats, mongooses and the black rat, should be implemented in this area and in peripheral surrounding areas in order to support survival of this small population of spiny rats. On Amami-Oshima Island, although the population of Amami spiny rats was severely deleted by feral dog (Watari et al. 2007) and mongoose predation (Yamada and Sugimura 2004), the distribution of the Amami spiny rat has shown recent signs of recovery following measures against these predatory species (Ministry of the Environmental 2010).

It is possible that other small populations of spiny rats still exist in the northern part of Okinawa Island in addition to that identified in this study, particularly in forests dominated by the same tree species as Area I, namely *Castanopsis sieboldii*, *Lithocarpus edulis*, *Distylium racemosum* and *Schima wallichii*. Further study is necessary to elucidate the fundamental ecology of this species as an aid to its conservation; in particular, it will be essential to determine the distribution range, population size, and population genetics of the species, to identify negative factors that threaten survival, and to develop a captive breeding technique.

Monitoring of changes in distribution range and population size is important for conservation of endangered species. This study confirmed that a combination of monitoring via sensor cameras and trapping could be used to determine the presence and distribution of a species. However, we also suggest that it may be necessary to increase the numbers of individuals that can be captured; the current limit is based on the guidelines for use and collection of animals in research (Taxonomic Names

and Collections Committee in the Mammalogical Society of Japan 2009). At present, the maximum number of individuals that can be captured is limited to 5–10 individuals per year by the Agency for Cultural Affairs and the Ministry of the Environment. This somewhat low limit hinders study of the species, and makes it difficult to conduct scientific research of relevance to its conservation.

In conclusion, our study has shown the survival of a small population of the spiny rat in the wild. Nevertheless, our results do not alter the situation of the spiny rat with regard to its vulnerability to extinction. Continuous investigation and monitoring will be necessary to ensure conservation of the species.

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