THE GUNONG BENOM EXPEDITION 1967

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SYNOPSIS

Rattus rajah (Thomas) and *Rattus surifer* (Miller) are two medium-sized spiny rats occurring in Malaysian forests which have proved difficult to distinguish by morphological characters, including external appearance and skeletal anatomy. Several authors have in fact combined them as a single polymorphic species.

Ecological data seem to indicate that these rats belong to separate species. Breeding and behaviour studies support this view. Karyotype and serological studies provide conclusive evidence for the distinctness of the two species.

THE names *Rattus rajah* (Thomas) and *Rattus surifer* (Miller) are applied to two medium-sized spiny rats occurring in Malaysian forests. The two taxa are phenetically rather alike and published opinions on their respective status differ. Bonhote (1903) regarded them as separate species belonging to the subgroup *rajah* of the *jerdoni* group. Chasen (1940) and Sody (1941), the principal revisers, retained both as full species. No firm opinion was offered by Tate (1936, 1947): "There appears to be a very strong resemblance between the many forms of the *rajah* group. One suspects that some will at length be shown as merely seasonal or age phases of single races". Ellerman (1949) attributed populations from Malaya to separate species under the names *R. rajah pellax* and *R. surifer surifer*. Ellerman and Morrison-Scott (1951), however, revised this opinion, and later (1955) suggested that *R. rajah* and *R. surifer* were probably conspecific. Harrison (1957, 1966) was inclined to take the same view: "This group is commonly divided into two forms, which may be called *R. rajah* and *R. surifer*, but there is some doubt about their being true distinct species. . . . Decision on the taxonomic question must be deferred until genetical and other studies are completed." Hill (1960), however, maintained that the two forms represented distinct species and clearly described the external features distinguishing them.

Although there is now no doubt that these rats can be readily distinguished by morphological characters (cf. Harrison, 1966), their systematic position, whether distinct species or a single polymorphic species, remains unsolved. It is the aim of this paper to provide an answer from various disciplines of comparative biological studies.

MATERIALS AND METHODS

All the rats used were trapped from the wild. Representatives of both were taken on Gunong Benom between 700–2500 ft, and additional specimens were collected

at the following localities: Kampong Janda Baik, Bentong District, Pahang; 19th mile Genting Simpah and Bukit Lagong, Selangor; Maxwell's Hill, Taiping, Perak; Kaki Bukit, Perlis. Trapping was carried out in a variety of natural habitats and the field data recorded. Collectors's flesh (body) measurements and measurements of the cleaned skull of 21 *R. rajah* and 19 *R. surifer* were analysed statistically; differences between means were tested for significance by "Student's" *t*-test. This "classical" taxonomic approach was augmented by genetical studies including karyology and serology. Breeding experiments were also set up to test the crossability of these rats.

RESULTS

External morphology. Despite extensive overlap in all body dimensions (Table 1), the sample of *R. surifer surifer* shows a significantly greater mean head and body length ($0{\cdot}05 > P > 0{\cdot}02$), mean tail length ($0{\cdot}01 > P > 0{\cdot}001$), and mean hind foot length ($P < 0{\cdot}001$). The mean ear length, however, is not significantly different from that of *R. rajah pellax* ($0{\cdot}3 > P > 0{\cdot}2$).

Table 1

Body dimensions of *RATTUS RAJAH* and *RATTUS SURIFER*

	Head & Body Length			Tail Length		
Species	Mean ± S.E. of Mean	C of V (%)	Obs. Range	Mean ± S.E. of Mean	C of V (%)	Obs. Range
R. rajah	174·29 ± 3·54	9·30	150–210	165·25 ± 3·88	10·51	132–200
R. surifer	187·32 ± 4·24	9·86	155–212	185·69 ± 4·33	9·32	157–215

	Hind Foot Length			Ear Length		
Species	Mean ± S.E. of Mean	C of V (%)	Obs. Range	Mean ± S.E. of Mean	C of V (%)	Obs. Range
R. rajah	37·86 ± 0·42	5·03	34–40	21·95 ± 0·71	4·66	20–23
R. surifer	42·21 ± 0·42	4·30	39–45	23·11 ± 0·68	4·05	22–25

	T : H & B (%)		
Species	Mean ± S.E. of Mean	C of V (%)	Obs. Range
R. rajah	94·91 ± 1·30	6·13	82·11–105·81
R. surifer	100·18 ± 1·57	6·26	91·51–112·12

S.E. = standard error
C of V = coefficient of variation
All measurements in millimetres

The pelage and tail colouration have been described in detail by Harrison (1957, 1966), Hill (1960) and Medway (1969). The two species are most readily distinguished by the presence in *R. surifer* of a sharp demarcating stripe on the flanks, formed by orange-tipped spines, and by the general body colouration.

Skull anatomy. Various skull dimensions are given in Tables 2, 3 and 4. Of these only the mean length of anterior palatine foramina and the mean length of upper molar series between these rats differ significantly ($P < 0.001$). There are

Table 2

Skull dimensions of *RATTUS RAJAH* and *RATTUS SURIFER*

	Bulla Length			Palatal Length		
Species	Mean ± S.E. of Mean	C of V (%)	Observed Range	Mean ± S.E. of Mean	C of V (%)	Observed Range
R. rajah	4·76 ± 0·07	5·59	4·40–5·20	19·88 ± 0·41	7·94	17·80–23·45
R. surifer	4·79 ± 0·07	6·30	4·30–5·40	20·77 ± 0·27	5·40	17·35–22·60

	L. of Ant. Palatine Foramina		
	Mean ± S.E. of Mean	C of V (%)	Observed Range
R. rajah	5·94 ± 0·10	8·73	4·90–6·80
R. surifer	6·64 ± 0·09	8·07	5·35–7·70

All measurements in millimetres
C of V = Coefficient of Variation

Table 3

Skull dimensions of *RATTUS RAJAH* and *RATTUS SURIFER*

	Occipitonasal Length			Length of Upper Molar Series		
Species	Mean ± S.E. of Mean	C of V (%)	Observed Range	Mean ± S.E. of Mean	C of V (%)	Observed Range
R. rajah	41·81 ± 0·81	7·22	37·70–48·20	6·82 ± 0·06	3·25	6·40–7·10
R. surifer	44·19 ± 0·57	5·45	37·40–48·20	6·47 ± 0·06	3·64	6·05–6·90

Species	Incisor-Molar L. / Palatal L.	Roots of Upper Molars
R. rajah	1	4,4,3;3,4,3;3,3,3
R. surifer	1	4,4,3;3,4,3;3,3,3

All measurements in millimetres
C of V = Coefficient of Variation

TABLE 4

DIMENSIONS OF SUBGENERIC CHARACTERS OF *RATTUS RAJAH* AND *RATTUS SURIFER*

		Bulla : Occ-nasal Length (%)		
Subgenus	Species	Mean ± S.E. of Mean	C of V (%)	Observed Range
Lenothrix	*R. rajah*	11·44 ± 0·14	4·90	10·42–12·27
	R. surifer	10·86 ± 0·16	6·26	10·05–12·83

		Palatal : Occ-nasal Length (%)		
Subgenus	Species	Mean ± S.E. of Mean	C of V (%)	Observed Range
Lenothrix	*R. rajah*	47·48 ± 0·32	2·65	44·65–49·48
	R. surifer	46·71 ± 0·91	1·63	45·02–48·34

		A.P.F. : Occ-nasal Length (%)		
Subgenus	Species	Mean ± S.E. of Mean	C of V (%)	Observed Range
Lenothrix	*R. rajah*	14·37 ± 0·18	6·87	12·76–16·14
	R. surifer	15·07 ± 0·15	5·83	13·20–16·72

Occ-nasal = Occipitonasal
A.P.F. = Anterior Palatine Foramina
C of V = Coefficient of Variation

no absolute differences between *rajah* and *surifer* in any of these characters. Other features of the skull such as general shape, shape of anterior palatine foramina, length of diastema, nasals, etc., also fail to demonstrate any morphological distinction.

HABITAT. *Rattus rajah* and *R. surifer* are found in the same general habitat, dipterocarp forests,* but are not normally present together (cf. Chasen, 1940). They occupy burrows in the ground, the entrances of which are loosely plugged with leaves. Trapping in Kampong Janda Baik, Genting Simpah, Maxwell's Hill, and Kaki Bukit produced only *R. surifer*, while trapping in Bukit Lagong and Gunong Benom yielded predominantly *R. rajah*. Only one *R. surifer* was trapped on Gunong Benom, where it was sympatric with *R. rajah* at an altitude of 2500 ft; 11 specimens of *rajah* were collected at this and lower altitudes.

BREEDING AND BEHAVIOUR. Both inter- and intra-specific crosses of *R. rajah* and *R. surifer* were unsuccessful under the prevalent animal-house conditions.

*Characterized by the presence of trees belonging to the family Dipterocarpaceae; forest floor hard and carpeted with fallen leaves; base of trees without crevices; and typically with three-storied canopy.

In intra-specific pairs, the rats settled down with their mates immediately. By contrast, in interspecific pairs, when first brought together the rats either ignored their partner or reacted agonistically. No mating attempt was observed in either inter- or intra-specific pairs.

In interaction experiments, where at least two freshly-trapped members of the same species were introduced at the same time into the cage, individuals of the same species aggregated together, irrespective of sex. When new individuals were introduced after the original members had established themselves, these newly-introduced individuals sought out members of their own species and joined them in a common shelter. When the newly-introduced individuals belonged to an unrepresented species, they occupied fresh shelters and did not mix with the other species.

Although a surplus of jars serving as shelters (Figure 1) was provided, rats of the same species always aggregated together, leaving the other jars unoccupied. Even after being kept together for at least six months, the two species failed to mix (*cf.* Yong, 1970).

KARYOLOGY. The karyotypes of both *R. rajah* and *R. surifer* have been described (Yong, 1969). The chromosome number (2n) and the number of chromosome arms (N.F. or N.A.) are distinctly different. The sex chromosomes (both X and Y) in both, however, are of the same morphological type. The karyotypes of these rats are summarised in Table 5. The karyological differences cannot be explained in terms of polymorphism, and indicate that two disinct species are represented.

TABLE 5

KARYOTYPES OF *RATTUS RAJAH* AND *RATTUS SURIFER*

	Number of specimens examined		2n	Pairs of autosomes*			Allosomes		
Species	male	female		S	A	M	X	Y	FN**
Rattus rajah pellax	2	1	36	3	8	6	M	M	56
Rattus surifer surifer	2	2	52	2	19	4	M	M	66

*S = subterminal, A = acrocentric, M = metacentric
**FN = Fundamental Number (number of chromosome arms)

SEROLOGY. The one-dimensional starch-gel electropherograms for *rajah* and *surifer* sera and haemoglobins are shown in Figures 2 and 3. The albumin band of *R. rajah* moves at a slightly slower rate than that of *R. surifer* under the same experimental conditions (Figure 2), but is nevertheless species-specific. In addition, *R. rajah* possesses a pre-albumin band of constant mobility, which is absent in *R. surifer*. *R. rajah* also differs from *R. surifer* in the possession of an extremely slow-migrating band, just next to the point of application of serum sample.

R. rajah and *R. surifer* also differ in their haemoglobin electrophoretic patterns (Figure 3). *R. surifer* possesses two rather faint cathodal bands and one or two dark-staining anodal bands. *R. rajah*, on the other hand, possesses two well-defined anodal bands, and one or two rather faint cathodal bands. The cathodal bands in *rajah* seem to be polymorphic.

The faster haemoglobin band in *rajah* moves with a mobility slightly slower than its albumin band. The two anodal bands are species-characteristic, and are faster migrating than the anodal bands characteristic of *surifer*.

The species-specific albumin pattern of one dimension starch gel-electrophoresis is also borne out in agar-gel immunoelectrophoresis, employing anti-*sabanus* as well as anti-*muelleri* sera produced in rabbits. Species-characteristic reaction patterns are observed in *R. rajah* and *R. surifer* (Figure 4).

DISCUSSION

Although external morphological characters, such as those distinguishing *surifer* and *rajah*, are reliable for identification, they are not so useful in assessing relationship. "There are . . . a few kinds of characters, notably those of colour and colour pattern, that are almost always highly labile wherever encountered" (Simpson, 1961). However, the experimental evidence presented above shows that *R. rajah* and *R. surifer* exhibit species-characteristic behaviour. Members of *R. rajah* associated and did not mix with *R. surifer* under simulated natural conditions; and the same was true of *R. surifer*. In both cases, the rats consistently huddled together although excess shelters were available, and there was opportunity for them to separate into isolated individuals or pairs. No misassociation was observed. This behaviour under wild conditions would keep the rats distinct when present in the same locality. The failure to hybridize in the laboratory cannot be interpreted as strong support, since intra-specific pairings were equally unproductive.

Karyological and serological studies furnish further concrete evidence. The karyotypes and the serum-protein and haemoglobin electrophoretic patterns of *R. rajah* and *R. surifer* are species-specific. The marked karyotype differences between *R. rajah* and *R. surifer* would be effective in preventing hybridization. The multitude of proteins would ensure genetic incompatibility and hence effectively keep these rats distinct.

In sum, the evidences from external morphology, skull anatomy, ecology, breeding and behaviour studies, karyology, and serology verify the species status of *R. rajah* and *R. surifer*. These rats belong to distinct valid species and are not colour varieties of a single polymorphic species.

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PLATE 1

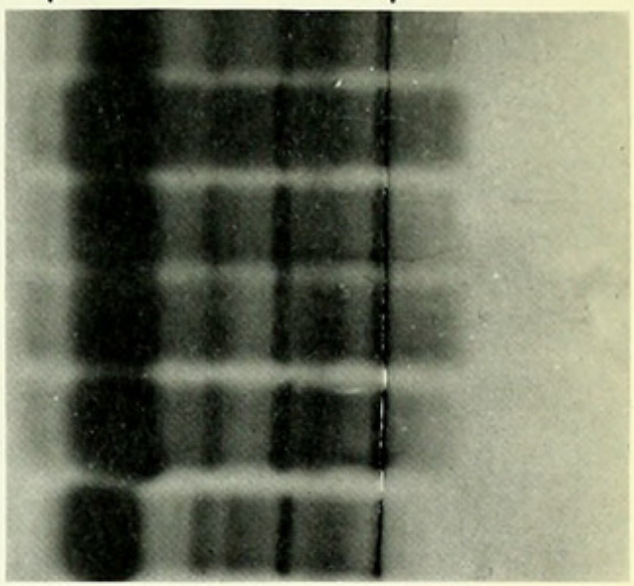
FIG. 1. Glass jars provided as shelters (nesting site) for rat.

FIG. 2. One-dimension starch-gel serum electropherogram of *Rattus rajah* compared with *Rattus surifer* (294S). Borate buffers were used; gel buffer with pH 8·8 and bridge buffer with pH 8·3. Electrophoretic run : 18 hrs. at 100 volts. Stained with amido-black.

FIG. 3. One-dimension starch-gel haemoglobin electropherogram of *R. surifer* (GS1, 11·7S) and *R. rajah* (270R, BLR, 312), compared with *rajah* serum (222R). Electrophoretic run as in Fig. 2.

FIG. 4. Serum immunoelectropherogram of *R. rajah* and *R. surifer* against anti-*sabanus* serum.

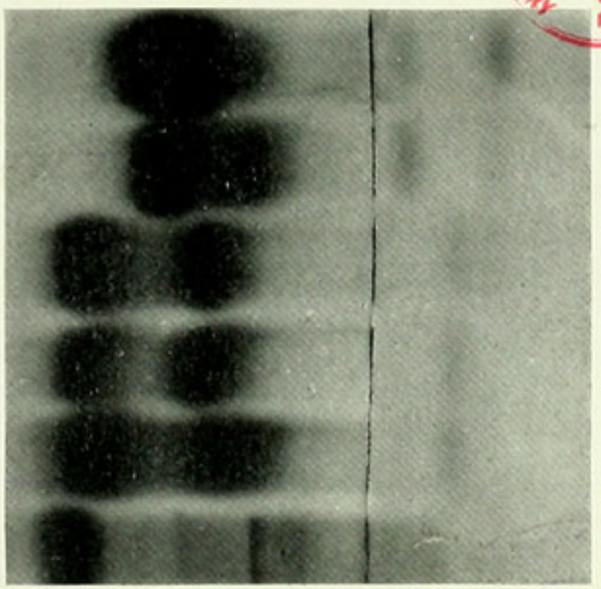
1



BLR$_1$ ♀
222 R ♂
292 R ♂
311R ♂
312 R ♂
294 S

2

GS$_1$ ♂ Hb
11.7 S ♂ Hb
270R ♂ Hb
BLR$_1$ ♀ Hb
312 ♂ RHb
222R ♂



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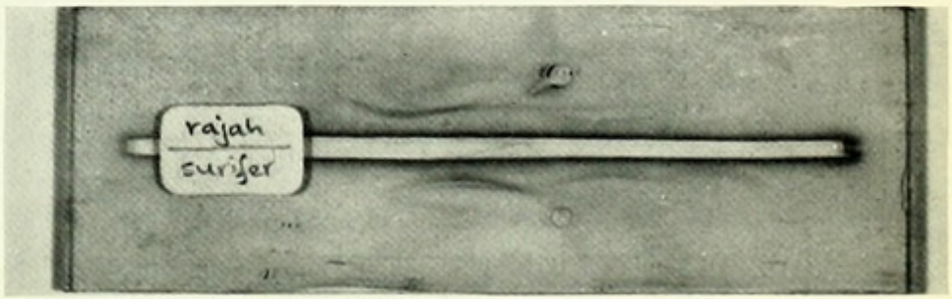

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